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EFFECTS OF PROGRESSIVE BETA CELL DYSFUNCTION BY REPEATED LOW DOSES OF STREPTOZOTOCIN ON INSULIN SENSITIVITY AND GLUCOSE EFFECTIVENESS IN DOGS

BY

BONNIE L. TOBIN

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science.

DEPARTMENT OF PHYSIOLOGY

Edmonton, Alberta
Spring 1992

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Bonnie L. Tobin

896 White Farm Road
Indiana, Pennsylvania 15701
United States of America

February 24, 1992

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled **EFFECTS OF PROGRESSIVE BETA CELL DYSFUNCTION BY REPEATED LOW DOSES OF STREPTOZOTOCIN ON INSULIN SENSITIVITY AND GLUCOSE EFFECTIVENESS IN DOGS** submitted by **BONNIE L. TOBIN** in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE**.

Diane T. Finegood
D.T. Finegood (Supervisor)

EJ Sanders
E.J. Sanders (Chairperson)

C.I. Cheeseman

P.K.T. Pang

E.A. Ryan

February 19, 1992

Dedicated to the memory of
Mary L. Schaeffer, my dear mother.

ABSTRACT

To investigate the longitudinal relationship between progressive beta cell dysfunction and other parameters of glucose tolerance, repeated low doses of streptozotocin (10 mg/kg body wt) were administered to canines at 14 day intervals. Streptozotocin treatments were discontinued following 10 doses (n=4), the onset of fasting hyperglycemia (n=1), or death (n=1). Prior to the initial streptozotocin dose and 8 days following each subsequent dose, an insulin modified frequently sampled intravenous glucose tolerance test (FSIGT) was performed. The integrated insulin response to glucose (IIRg, 0-19 minutes), the acute insulin response to glucose (AIRg), insulin sensitivity (S_I), glucose effectiveness (S_G), and glucose tolerance (K_G) were determined from this protocol. Two days subsequent to each FSIGT, a slope of potentiation protocol was performed to determine the acute insulin response to arginine (AIRarg) at basal glycemia, the AIRarg at hyperglycemia, and the slope of potentiation of the AIRarg (slope). A total of 105 experiments were performed.

The integrated insulin response to glucose declined gradually, reaching a nadir equal to 13% of pre-treatment values by completion of the study. Despite this significant reduction in the insulin secretory response to glucose ($p=0.02$), fasting plasma insulin, fasting plasma glucose, and S_I remained constant throughout the study period ($p=0.15$, $p=0.71$, and $p=0.5$ respectively). S_G, however, declined to 45% of pre-streptozotocin values in animals who received more than 7 streptozotocin doses (>70 mg/kg streptozotocin cumulatively, $p<0.03$).

The relationships between the AIRg from the FSIGT and the indicators of insulin secretory function from the slope of potentiation protocol were determined at each streptozotocin dose level. The AIRg significantly correlated with all three measures of secretion derived from the slope of potentiation protocol (AIRg vs. AIRarg at basal glycemia, r=0.87; AIRg vs. AIRarg at hyperglycemia, r=0.93; AIRg vs. slope, r=0.83; all p values = 0.0001).

In summary, when fasting normoglycemia is maintained, an 86 - 93% reduction in insulin secretory capacity by repeated low doses of streptozotocin may alter glucose effectiveness, but do not directly affect insulin sensitivity. In addition, the acute insulin response to glucose from the FSIGT is equivalent to the slope of potentiation as a measure for quantification of beta cell function.

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I. INTRODUCTION AND LITERATURE REVIEW

A. GENERAL INTRODUCTION

The overall objective of this thesis is to elucidate the metabolic alterations associated with diminished insulin secretory capacity. More specifically, this research was performed to determine the effects of a progressive decline in insulin secretion on insulin sensitivity, glucose effectiveness, and glucose tolerance.

In diabetes mellitus, glucose tolerance is moderately or severely impaired. Diabetes, however, is not one discrete disease, but is a heterogenous disease. The two major types of diabetes are Type I diabetes mellitus (insulin dependent diabetes mellitus, IDDM) and Type II diabetes mellitus (non-insulin dependent diabetes mellitus, NIDDM). The etiologies of these two types of diabetes are distinct and classification of the diseases is based upon their specific clinical features.

Insulin dependent diabetes is a genetically determined chronic autoimmune disease in which primary beta cell destruction occurs prior to the manifestation of clinical symptoms (46, 60, 125). In fact, pathogenesis may precede the overt onset of the disease by several years (60, 125). Environmental factors are believed to be involved in the "triggering" of the autoimmune process, though it is not known if such factors are essential for IDDM development (43). By the time a clinical diagnosis is made, the autoimmune destruction of the beta cells is extensive, though some insulin secretory function remains (45) (47, 92, 123). Ultimately, progression of the disease leads to complete and irreversible beta cell destruction (46).

In the past, IDDM was considered to be a disease exclusively involving the pancreas. It was recognized at that time that the beta cells of the islets of Langerhans cannot synthesize and secrete adequate quantities of insulin to maintain normal blood glucose concentrations. However, it is now known that in overt IDDM, loss of beta cell function is accompanied by insulin resistance, i.e. a decrease in insulin sensitivity (17, 33, 42, 117, 154). Insulin sensitivity is the ability of the insulin target tissues to enhance glucose disappearance in response to the circulating hormone. Studies have been performed to determine whether the development of insulin resistance in IDDM is mediated via insulin deficiency per se or if a secondary metabolic derangement such as hyperglycemia is responsible. No consensus has been reached yet, as the literature supports both possibilities (22, 50, 52, 78, 98, 100, 102, 118, 135, 137).

In contrast to IDDM, non-insulin dependent diabetes mellitus has generally been characterized by a decrease in tissue insulin sensitivity, which is believed to be due to alterations in the insulin receptors or some process beyond the receptors (10, 32). Current knowledge, however, indicates that like insulin dependent diabetes, NIDDM is also characterized by both insulin resistance and a reduction in insulin secretory capacity (141, 142, 143). It remains unclear which abnormality precedes the other, decreased sensitivity or decreased secretion. Without the presence of some impaired insulin secretion, insulin resistance by itself does not generally produce significant hyperglycemia (141). Both insulin resistance and diminished insulin secretion must be present simultaneously before moderate to severe glucose intolerance is detected (41).

Thus, it is evident that there are degrees of decreased insulin secretion and insulin sensitivity in both IDDM and NIDDM. The present thesis will predominately focus on the investigation of metabolic changes associated with a primary deterioration of insulin secretory function. The relationships between progressive beta cell dysfunction and the relative contribution of alterations in the other factors determining glucose tolerance, are not well understood. A better understanding of these relationships may facilitate the development of intervention programs which could improve the prognosis in pancreas/islet cell transplantation, prolong remission from diabetes, or delay the onset of clinical IDDM.

B. FACTORS DETERMINING GLUCOSE TOLERANCE

Glucose tolerance is the ability to dispose of an oral or intravenous glucose load. This capacity to "tolerate" glucose depends not only on the rate of appearance of insulin, but also on 1) the rate at which insulin is cleared from the plasma, 2) the sensitivity of tissues to insulin's action (S_I), 3) the ability of glucose itself to enhance its own disposal (S_G) and 4) in the case of oral glucose, the rate of glucose absorption across the gut (17).

Insulin Secretion

Insulin is a hormone which is secreted from clusters of endocrine cells in the pancreas, known as the islets of Langerhans. There are 100,000 to 2,500,000 islets per pancreas (38). The islets contain four distinct types of cells; the alpha cells which secrete glucagon, the delta cells which secrete somatostatin, the F cells which secrete pancreatic polypeptide, and the beta cells which are the site of insulin synthesis and secretion. The beta cells are the most abundant of the islet endocrine cells, comprising from 70-90% of the islet cell mass (38, 55).

In the beta cell, insulin is synthesized from the precursor proinsulin (104), a spiral shaped molecule consisting of an A and B chain linked by a connecting peptide (C-peptide). The proinsulin is cleaved by proteases to yield equimolar amounts of insulin (A and B chains connected by disulfide bridges) and C-peptide, which are then packaged into secretory granules (79). The mature granules migrate towards the plasma membrane. Upon arrival at the cell surface, the membrane of the secretory granule fuses with the plasma membrane to release the granule contents into the extracellular space, a process known as exocytosis (69). Since insulin and C-peptide are secreted from the granule in equimolar amounts (57), both can be used to assess beta cell function. However, the metabolic clearance rate of C-peptide is much slower than that of insulin. For this reason, C-peptide values may be used to indicate insulin reserve (79), but not insulin dynamics.

In contrast to C-peptide, plasma insulin concentrations may be used to study insulin dynamics and are more frequently used to evaluate insulin secretory capacity. The rate limiting step in the insulin secretory process is associated with the hormone release into the general circulation from the secretory granules, i.e. exocytosis (38). Under normal conditions there are two phases of insulin release. The first phase insulin release is the acute response to a secretogogue which is thought to represent the secretion of prestored insulin (39) from granules that are adjacent to the beta cell membrane (38). This immediate response subsides within about ten minutes. In contrast, the second phase insulin release is characterized by a gradual secretion of insulin in response to a secretogogue (39). This phase is believed to be the release of newly synthesized insulin granules (38, 57, 58). Vesicles containing the "new" insulin are released into the cellular cytosol by budding off from the golgi apparatus (79) and may follow a direct route from the interior of the cell to the plasma membrane (57) for immediate secretion into the circulation. Thus, in contrast to the older insulin granules which are stored adjacent to the plasma membrane, the newly formed granules which are released spontaneously are considered to be more "internal". During continuous glucose stimulation, the more recently formed granules are secreted first (57, 58), while older granules accumulate and may eventually be broken down via lysosomes for recycling (104).

Once insulin is secreted into the blood, it passes into the portal venous system, resulting in high insulin concentrations in the liver. The liver, however, degrades and clears 40 - 60% (79) of the insulin (57), so that the plasma concentrations in the peripheral tissues are lower. The insulin that passes beyond the liver has two principle target sites, adipose and muscle tissue. Under normal conditions these tissues are dependent upon insulin's action to promote glucose metabolism, and are therefore considered to be insulin "sensitive" tissues.

Insulin Sensitivity

Insulin sensitivity (S_I) is a measure of the effect of insulin concentrations above the basal level to enhance glucose disappearance. Historically, in 1939, Himsworth and Kerr first demonstrated two categories of individuals with diabetes; insulin-sensitive and insulin-insensitive (67). The insulin-sensitive diabetics tended to be younger, thinner, and prone to ketosis, while the insulin-insensitive individuals generally were older, usually obese, and nonketotic. These two original classifications of diabetes correspond to what is currently identified as Type I and Type II diabetes. Today, however, it is well established that there is a degree of insulin resistance associated with not only non-insulin dependent diabetes, but also with the severely impaired insulin secretion in IDDM (17, 33, 42, 117, 154). By the onset of clinical IDDM, insulin sensitivity is reduced by approximately 32 - 61% (42, 102). With initiation of exogenous insulin treatment, insulin action increases towards normal, but after a few months of therapy, insulin resistance reappears (10). The principle sites of this resistance are the liver and muscle, but quantitatively skeletal muscle plays the most important role in altering glucose homeostasis.

At the target tissue, insulin's action begins with the binding of the hormone itself to specific cell surface receptors. It is believed that the principle role of the insulin molecule is to alter the conformation of the insulin receptor resulting in an active ligand/receptor complex (63). After binding occurs, the effect of insulin to activate glucose transport under normal conditions is rapid. Insulin binding, however, is not considered to be the rate limiting step for glucose metabolism. Instead, the rate limiting step is believed to occur subsequent to the transport of glucose into the cell (10).

Under normal conditions insulin stimulation promotes an increase in the number of functional glucose transporters at the plasma membrane of insulin sensitive tissues (129, 145, 146). This increase in glucose transporter number results from recruitment or translocation of transporter rich vesicles from an intracellular site to the cell surface (40). The translocation of glucose transporters to the plasma membrane, however, cannot account for all of the effect of insulin on glucose transport (72). It appears that the insulin has an additional effect to induce conformational changes in the transporters, which alters the activation energy of transport (72). Thus, a portion of the augmented glucose transport activity in response to insulin may include an increase in the transporter turnover rate or the number of glucose molecules a single transporter can carry across the plasma membrane.

Further research will be necessary to fully understand the normal mechanism by which insulin binding stimulates glucose transporter translocation and increases the activation energy of transport. With regards to diabetes, it has been shown in diabetic animals, that the number of glucose transporters at the cell surface are greatly reduced in response to insulin stimulation (13, 76). The association of this abnormality with the primary defect in IDDM remains to be clarified. As well, the turnover rates for the insulin sensitive glucose transporters under normal and diabetic conditions, are yet to be defined.

Glucose Effectiveness

Another determinant of glucose tolerance is glucose action or the effect of glucose itself to promote its own disposal through uptake by mass action into the tissues and through suppression of endogenous glucose production, at basal insulin. Glucose effectiveness (S_G) is a quantitative measure of glucose action, representing the net increase in glucose disappearance for each increment in glucose concentration. Thus, it is a combined measure of the effects of glucose on both the rate of appearance (production) and the rate of disappearance (uptake) of glucose.

The first hint that not only insulin, but glucose itself has an effect on glucose tolerance, came from a study performed by Soskin et al. in 1934 (124). At this time, abnormal glucose tolerance was believed to be totally caused by a lack of the pancreatic insulin response. Soskin and his colleagues performed dextrose tolerance tests on normal and depancreatized dogs as a means to test this hypothesis and in attempts to clarify the mechanisms involved in maintaining a normal dextrose tolerance curve. During these experiments a constant infusion of insulin was administered to the depancreatized dogs, without a compensatory increase in the infusion during dextrose administration. In this original study, it was demonstrated that totally depancreatized dogs exhibited normal "tolerance" to intravenous dextrose when compared to control animals. These results opposed the concept of that era by providing evidence that the normal dextrose tolerance curve is not entirely dependent upon insulin secretion from the pancreas. They then investigated the liver's role in glucose tolerance by performing hepatectomies with and without concurrent pancreatectomies. This study was the first to demonstrate that with an intact pancreas, when the liver is absent, an abnormal dextrose tolerance curve ensues. Furthermore, it was determined that the means by which the normal liver contributes to glucose tolerance is by decreasing its own glucose output in response to elevated plasma glucose. Thus, the work of Soskin et al. provided the first evidence that the hepatic response to plasma glucose concentration plays a major role in glucose tolerance. This effect of glucose to suppress hepatic glucose production is one component of what is now termed "glucose effectiveness".

Since the pioneering work of Soskin et al. (124), research pertaining to the action of glucose on hepatic glucose balance, has been limited. Investigations in the 70's by Bergman and Bucolo (16) and Bucolo et al. (31) determined that in addition to the role of glucose in the suppression of hepatic glucose production, glucose itself also regulates it's own uptake into the liver during feeding. Further progress in this area, however, was hindered by the fact that it was impossible to suppress insulin secretion during hyperglycemia. It wasn't until somatostatin became available, that the role of hyperglycemia per se in the regulation of hepatic glucose production was further examined. When somatostatin was used to suppress endogenous insulin and glucagon secretion, the conclusion of Soskin et al. (124) was confirmed (90). In 1979, Liljenquist et al. demonstrated that hyperglycemia itself results in significant inhibition of splanchnic glucose production in man (90). These early investigations indicated that quantitatively, glucose per se plays a major role in the regulation of net hepatic glucose balance.

In addition to the role of glucose in the suppression of hepatic glucose production, another function of glucose is to increase it's utilization in the peripheral tissues. In 1978, Cherrington et al. performed a study in dogs which provided evidence that in the presence of unchanging basal amounts of insulin and glucagon, the disappearance rate of glucose from plasma increases almost proportionally to an increase in plasma glucose concentration (37). Results from this study indicated that glucose itself has an effect to enhance glucose disappearance at the peripheral tissues. Two subsequent studies by Best et al. (19) and Verdonk et al. (139) confirmed in humans, what Cherrington and his colleagues (37) observed in dogs.

In their investigations, Best et al. (19) and Verdonk et al.(139) examined the relationship between plasma glucose concentration and insulin independent glucose disposal in humans. In both studies, glucose clamp techniques were used to determine glucose utilization at various steady state glucose concentrations. During these studies, endogenous insulin secretion was suppressed via somatostatin, while exogenous insulin infusions were used to maintain constant plasma insulin levels. Results from these investigations demonstrated that glucose utilization is enhanced when plasma glucose is elevated, even when insulin concentrations remain unchanged. At plasma glucose concentrations above 60 mg/dl, glucose utilization was found to be virtually proportional the plasma glucose level. In contrast, at low plasma glucose concentrations (0 - 60 mg/dl), the increase in glucose utilization was not proportional to a given increment in the plasma glucose level, such that utilization was greater than the elevation in plasma glucose. They suggest that this phenomenon may occur because the non-insulin-mediated glucose uptake in the brain is not substantially affected by alterations in glycemic levels. Thus, at hypoglycemic levels, when other tissues are sparing glucose, the brain is the major consumer of glucose. At normoglycemic or hyperglycemic levels, other tissues participate in non-insulin-mediated glucose uptake, accounting for the proportional elevations observed in glucose utilization. These two investigations have contributed significantly to our understanding of the role of glucose itself in maintaining glucose homeostasis. This action of glucose to promote it's own uptake at the peripheral tissues is the second component of glucose effectiveness.

Thus, it is now understood that glucose itself can inhibit hepatic glucose production and increase glucose utilization. In normal healthy individuals, it has been demonstrated that this non-insulin-mediated glucose uptake accounts for 75-85% of total glucose uptake in the postabsorptive state (61). In subjects with insulin dependent diabetes, it has been suggested that glucose effectiveness is decreased (50). However, since only limited attention has been focused on the action of glucose per se, the natural history of the development of impaired glucose effectiveness remains to be defined.

C. METHODS OF ASSESSING THE FACTORS DETERMINING GLUCOSE TOLERANCE

Insulin Secretion

Oral Glucose Stimulation

In an oral glucose tolerance test (OGTT) a specific glucose load is administered orally to assess the adequacy of the insulin secretory response to return plasma glucose to basal concentrations. It is difficult, however, to use this method as a measure of beta cell function per se, since variations in the rate of glucose absorption across the gut and the effects of gastrointestinal hormones must be considered. These influences alone can alter plasma glucose concentrations and consequently, the rate of beta cell stimulation. Thus, the OGTT may be used to determine glucose tolerance, but is not considered to be an appropriate tool for accurately assessing isolated beta cell function (38). Additionally, it is not suitable for most animal experimentation.

Intravenous Glucose Stimulation

An intravenous glucose tolerance test (IVGTT) is a clinical test to determine plasma glucose and insulin dynamics subsequent to an intravenous (IV) glucose injection. Generally only 3-9 blood samples are collected during a basic IVGTT. The frequently sampled IV glucose tolerance test (FSIGT) is a modification of the IVGTT in which a larger number of samples (20-30) are collected to determine plasma glucose and insulin dynamics. The FSIGT was designed to more accurately assess islet cell function and insulin sensitivity from the response to a bolus of intravenous glucose (15).

From the FSIGT two measures of insulin secretion can be calculated, the integrated insulin response to glucose (IIRg) and the acute insulin response to glucose (AIRg). The integrated insulin response to glucose is estimated by calculating the area under the insulin concentration curve, above basal, following the glucose injection. The AIRg is determined by subtracting the basal insulin concentration from the mean insulin concentration in the 2-5 minutes post glucose injection.

There are several advantages of using the FSIGT to determine insulin secretory function. First, the prevailing plasma glucose concentration does not have a potentiating effect upon the AIRg (i.e. the first phase insulin response), as is the case with most other secretagogues (38). The first phase insulin response to glucose does not rely on newly formed insulin, but instead involves only the release of prestored insulin (39). In contrast, the second phase insulin release which as a result of being at least partially reliant on newly synthesized insulin (58), is dependent upon the prestimulus glucose concentration. When the level of glycemia is elevated in a continuous manner, newly synthesized insulin is preferentially released (39), preserving the "old" insulin granules that are available for an acute response to a rapid rise in the plasma glucose concentration. Thus, when the AIRg from the FSIGT is used to determine insulin secretion, it is not necessary to match basal glucose levels between subject groups.

A second advantage of determining insulin secretion from the FSIGT is that in contrast to the OGTT, there is no effect of gastrointestinal abnormalities. Thirdly, several parameters can be derived from the FSIGT. Briefly, the integrated insulin response to glucose (IIRg), the acute insulin response to glucose (AIRg), glucose tolerance (K_G), insulin sensitivity (S_I), and glucose effectiveness (S_G) can all be determined from this one protocol. These measurements will be reviewed in subsequent sections of this thesis.

Intravenous Arginine Stimulation

Arginine, an amino acid, is a potent secretagogue used to stimulate insulin secretion for characterization of beta cell function. Under conditions of normal glucose tolerance, the magnitude of the acute insulin response to arginine (AIRarg) is directly related to the circulating glucose concentration (62, 85). Thus, the effects of arginine are greater when glucose is infused to maintain an elevated plasma glucose concentration than when arginine is administered at basal glucose. If the effects from a non-glucose secretogogue were not dependent on the plasma glucose concentration, detrimental hypoglycemia could develop when the prevailing plasma glucose level is low.

Ward and colleagues (141) have termed the effect of hyperglycemia to enhance the acute insulin response (AIR) to a non-glucose secretagogue, "glucose potentiation". The slope of the relationship between the AIRarg and the plasma glucose level is known as the "slope of potentiation" (62). As glucose tolerance deteriorates, the insulin secretory response to arginine diminishes, precipitating a decline in the AIRarg and the slope of potentiation of the AIRarg (slope). Hence, both the AIRarg and the slope can be used as indicators of beta cell function. One disadvantage of the slope of potentiation is that a decrease in the slope could be caused by a decrease in the overall capacity of the pancreas to secrete insulin or a decrease in the "sensitivity" of the beta cells to respond to the potentiating effects of glucose (38, 75).

The slope of potentiation has been advocated by some (98) as being the most sensitive indicator of beta cell function. This suggestion is based on evidence which indicates that a loss of beta cell function impairs the glycemic potentiation of the insulin response to a non-glucose stimuli, such as arginine, more than it reduces the direct insulin response to glucose (98, 99, 144).

McCulloch and colleagues (98) compared the AIRg, the AIRarg, and the slope of glucose potentiation in baboons 1 and 8 weeks post streptozotocin administration (55 mg/kg, for 3 days). In their protocol an arginine bolus was administered at 0 minutes, a glucose injection was given at 30 minutes for determination of the AIRg, and approximately 3 hours later a second arginine pulse was given after raising the plasma glucose level to 14 mM via a continuous glucose infusion. At 1 week, the slope of potentiation indicated the most significant beta cell dysfunction. In comparison to pre-streptozotocin values, the AIRg had decreased by 62%, the AIRarg had decreased by 52%, and the slope had decreased by 94%. At 1 week insulin sensitivity was also reduced by 54% of the pre-streptozotocin value ($p<0.05$). By 8 weeks there were improvements in the AIRg and the AIRarg, such that they resumed levels which were not significantly different from pre-streptozotocin values. The slope of potentiation, however, remained impaired. Thus, the improvement in insulin secretion indicated by the AIRg and AIRarg was not reflected in the slope. Based on these results the authors concluded that the slope of glucose potentiation of AIRarg is the most sensitive indicator of beta cell damage.

Ward and colleagues (144) studied partially (two-thirds) pancreatectomized dogs using a protocol which was similar to that of McCulloch et al.'s (98), in that the AIRg was determined on the same day as the AIRargs. Ward and colleagues administered arginine 4 times for determination of the AIRarg (at 0, 75, 260, and at 335 minutes) and glucose was injected at 280 minutes for determination of the AIRg. Beginning at 30 minutes, a continuous variable-rate glucose infusion was administered to maintain a plasma glucose level of approximately 14 mM for determination of the AIRarg. The glucose infusion was stopped at 105 minutes to allow the plasma glucose to return to basal before the glucose bolus was injected for determination of the AIRg. At 290 minutes the glucose infusion was resumed to achieve a steady state plasma glucose level of approximately 33.6 mM. At six weeks post pancreatectomy they observed a 24% decrease in AIRg, a 47% decrease in AIRarg at basal, a 62% decrease in AIRarg at 14 mM, and a 75% decrease in AIRarg at 33.6 mM. Based on these results, they concluded that the reduction in the glycemic potentiation of the insulin response to arginine is the best means of detecting subclinical beta cell loss in two-thirds pancreatectomized dogs.

In conflict with the results of Ward et al. (144) and McCulloch et al. (98), an investigation in our laboratory concluded that islet cell mass is not well correlated with the slope of potentiation. Male Wistar Furth rats were made diabetic with streptozotocin and subsequently transplanted with 500, 1000, 2000, or 3000 islets (134). In this animal model of diabetes in which islet mass was pre-determined, the islet cell number was significantly correlated to the AIRg (r=0.53, p=0.02) and the AIRarg (r=0.50, p=0.02), but not the slope (r=0.36, p=0.11). This data supports the AIRg as being a better measure of beta cell function than the slope of potentiation.

An additional investigation in our laboratory compared the slope and the AIRg from the FSIGT in normal and islet transplanted canines (133). At these two levels of beta cell function (normal and 9-29% of normal), significant correlations were demonstrated between the AIRg and slope (r=0.89, p<0.0001) and also the AIRg and AIRarg (r=0.91, p<0.0001).

Validation of the AIRg across the spectrum of beta cell function would be advantageous, since the FSIGT not only provides a measure of insulin secretory capacity, but also allows determination of insulin sensitivity, glucose effectiveness, and glucose tolerance. Additionally, the FSIGT is more economical and is easier to perform than the slope of potentiation protocol.

Insulin Sensitivity

Glucose Clamp

The euglycemic glucose clamp is the most direct measure of insulin sensitivity and is therefore considered to be the "gold standard" which other measurements of insulin sensitivity have been validated against (4). The glucose clamp uses external feedback control to maintain the plasma glucose concentration at a constant basal level (euglycemic) via a variable rate glucose infusion, while a constant rate insulin infusion is maintained. Insulin's action to suppress glucose production and increase glucose utilization, is balanced by the glucose infusion (GINF) rate needed to maintain steady state glycemic conditions. Therefore, the GINF rate is a measure of tissue sensitivity to exogenous insulin.

A disadvantage of using the glucose clamp is that the measure of insulin sensitivity derived from it is dependent on the prevailing glucose concentration, so that the value obtained is not absolute (17, 51). It has been shown in the literature, however, that the glucose clamp can be used to derive a measure of insulin sensitivity which has the potential to be independent of the glycemic level at which it is determined (17, 51). Two methods have been proposed for "correcting" the insulin sensitivity value from the glucose clamp. First, instead of simply using the GINF to indicate insulin sensitivity, the ratio between the GINF and the steady-state increase in the plasma insulin concentration is used as an index of insulin sensitivity. This index is believed to be independent of the glucose level at which the insulin sensitivity is determined (51). The second method of "correction" used to derive a value of insulin sensitivity from the glucose clamp is based on the ratio of the increase in the rate of glucose disappearance to the change in insulin concentration normalized to plasma glucose (17). Values derived via either of these methods theoretically represent measures of insulin sensitivity which are independent of the glycemic level at which the parameter is determined. It is believed that these correction factors can be used with confidence within a physiologic range of glycemia (3.36 - 8.96 mM) (17, 51). Whether these relationships are maintained in pathophysiologic states has not been determined.

Minimal Model Method

The minimal model of glucose disappearance is a physiologically based model which is able to account for the glucose dynamics during a frequently sampled intravenous glucose tolerance test (FSIGT). The model assumes that there is a remote compartment into which plasma insulin enters. This compartment represents the delay in insulin action from its appearance in plasma to the time of its effect to promote glucose disposal and suppress hepatic glucose production.

MINMOD is the mathematical computer program which contains the minimal model of glucose disappearance used to determine insulin sensitivity and glucose effectiveness (107). Actual plasma glucose and insulin concentrations from an FSIGT are entered into the computer program. There are four constant parameters in the model: P_1 (glucose disappearance term, independent of insulin response, S_G), P_2 and P_3 (insulin distribution and metabolism parameters), and G_0 (glucose concentration at time zero, i.e. prior to the glucose bolus) (15). MINMOD then "guesses" a set of constant parameters, which is used along with the actual insulin data as input to solve the model equations, yielding estimates of plasma glucose. These estimated glucose values are compared to the real glucose data. Parameter coefficients are then updated in attempts to derive estimated glucose values which more closely resemble the actual data. Iterations of this process continue until the coefficients selected in association with the actual insulin data, result in estimates of plasma glucose which best describe or "fit" the actual plasma glucose values. The parameters which are identified with the best fit are then used to define glucose effectiveness ($S_G=P_1$) and insulin sensitivity ($S_I=P_3/P_2$) from the actual experimental data.

Finegood and colleagues compared the insulin sensitivity index from the FSIGT to the measurement of insulin sensitivity from the euglycemic glucose clamp in dogs (51). The MINMOD computer program (107) was used to estimate insulin sensitivity, as the increase in fractional glucose disappearance rate per unit increase in plasma insulin concentration, during the FSIGT. Results from this investigation demonstrated that the insulin sensitivity values from the glucose clamp and the minimal model method were highly correlated ($r=0.82$, $p<0.005$). Later, a modification of the original FSIGT was developed to ensure an endogenous insulin response of sufficient magnitude to measure insulin sensitivity (9, 153). In this modified FSIGT, tolbutamide, an insulin secretogogue, was injected 20 minutes after the glucose bolus (15). The tolbutamide modified FSIGT protocol, however, is not appropriate for use in subjects who do not have a sufficient endogenous insulin secretory response (148), as may be the case in IDDM. Therefore, tolbutamide was replaced by insulin in yet another modification of the FSIGT. In this experimental protocol, exogenous glucose and insulin are injected at 0 and 20 minutes, respectively. The insulin modified FSIGT has been validated for use in both humans (50) and dogs (49).

Glucose Effectiveness

Minimal Model Method

As was briefly described above, application of the MINMOD computer program to data from an FSIGT can be used to determine glucose effectiveness (S_G). The glucose and insulin dynamics following an intravenous glucose bolus are used to quantify S_G as the fractional clearance rate of glucose that would be observed in the absence of a dynamic insulin response to a glucose injection (i.e. at basal insulin). The MINMOD derived measure of S_G was validated in dogs by comparing it to a direct measurement of glucose action (2), i.e. glucose disposal measured during somatostatin suppression of the dynamic insulin response, with simultaneous intraportal infusion of insulin and glucagon to mimic basal, postabsorptive conditions. By comparing MINMOD derived values of S_G and direct measurements of glucose action, S_G has been determined to be an adequate representation of the interaction between glucose and glucose disappearance.

Insulin-independent Glucose Uptake

Gottesman et al. described a technique to determine insulin-independent glucose uptake (61). Glucose clamps, employing tritiated glucose, were used to determine total glucose uptake at three glucose concentrations (~60, 95, and 160 mg/dl). Somatostatin was infused to suppress endogenous insulin secretion while a constant infusion of insulin was simultaneously administered. On separate occasions, insulin was infused at three different rates (0.2, 1.0 and 2.0 $mU \cdot kg^{-1} \cdot min^{-1}$) at each glucose concentration. An insulin dose response curve was then completed at each glucose level. Using linear regression, the data from each dose response curve was extrapolated back to zero to derive the rate of insulin-independent glucose disposal. Determination of glucose action by this method, however, is not financially practical or time efficient. Additionally, this method assumes linearity of glucose uptake at insulin concentrations lower than those which can actually be studied.

Non-insulin-mediated Glucose Uptake

Baron et al. (7) studied non-insulin-mediated glucose uptake (NIMGU) by clamping glucose at a desired level during a period of insulin suppression induced by a somatostatin infusion. Glucose disposal was quantified during the glucose clamps by infusing tritiated glucose. This method is considered to be a direct measurement of NIMGU. In contrast to S_G, which is a measure of the effect of glucose on both glucose uptake and glucose production, NIMGU only accounts for the action of glucose on it's own uptake. Two additional differences between NIMGU and S_G are: 1) NIMGU is a steady state glucose flux, which is dependent on the prevailing glucose concentration, whereas S_G is a fractional rate constant measured under non-steady-state conditions and is independent of the glucose concentration and 2) NIMGU is measured during suppression of insulin, while S_G is derived at basal insulin. Two possible drawbacks to consider when using NIMGU as a measurement of glucose action are 1) it's reliance on the complete suppression of insulin by somatostatin and 2) the possible effect of somatostatin to promote glucose uptake (14).

Summary

In this section I have briefly summarized a variety of methods which can be used to assess insulin secretion, insulin sensitivity, and glucose effectiveness. Much research in the past has focused on the contributions and mechanisms of diminished beta cell function and insulin resistance in IDDM. Little research, however, has been performed to investigate the role and mechanism of glucose action during glucose intolerance or subclinical insulin deficiency. By gaining insight into the natural history and the underlying mechanism of impaired glucose effectiveness, we may develop a better understanding of the function of glucose action in the regulation of normal glucose homeostasis. Additionally, we will improve our comprehension regarding the role of impaired glucose action in the pathogenesis of diabetes and possibly improve the efficacy of therapeutic intervention.

D. SUBCLINICAL INSULIN DEFICIENCY

Presently, the term "subclinical insulin deficiency" will be used to refer to a condition in which beta cell function is adequate to maintain normal fasting plasma glucose, but the capacity of the beta cells to secrete insulin in response to glucose or other secretagogues is reduced. This situation is present in the pre-clinical stage of

IDDM and also during the "honeymoon" phase or remission from IDDM. The "honeymoon" phase is characterized by a relatively short period of improved glucose tolerance subsequent to the acute onset of IDDM. This brief remission generally follows the introduction of exogenous insulin therapy and is probably related to the improved insulin action and secretion associated with the correction of hyperglycemia (47). The primary focus of this thesis, however, will be related to changes in the parameters of glucose tolerance which occur during progressive beta cell dysfunction.

Eisenbarth described six hypothetical stages of IDDM (46). The first two stages are genetic susceptibility and the triggering event. Though not well defined, some possible "triggers" include environmental factors such as chemical agents, infectious agents, and viral agents which may promote defects in immune mechanisms. The third stage of IDDM described by Eisenbarth is active autoimmunity. During this stage when immunologic abnormalities are developing, insulin secretion may be normal. In stage four, however, glucose stimulated insulin secretion is progressively lost, despite normal blood glucose levels. Stage five then is marked by the onset of overt diabetes which is characterized by hyperglycemia. By this stage extensive beta cell destruction has occurred and will eventually lead to the last stage of the disease which is ultimately represented by complete beta cell destruction.

Insulin Secretion

The focus of the present thesis is the fourth stage of Eisenbarth's six hypothetical stages of IDDM (46), the time period immediately prior to overt diabetes. As stated above, this stage of pre-clinical diabetes is represented by the progressive loss of glucose stimulated insulin secretion (46). The beta cell loss which is characteristic of this phase can take place over several years prior to the manifestation of overt diabetes and is generally associated with a loss of the first phase insulin response to IV glucose (46, 47, 125). In contrast, the responses to IV glucagon, IV tolbutamide, IV arginine, and oral glucose, are apparently preserved (54, 126). Though a moderate impairment in insulin secretory function is evident during this pre-clinical phase of the disease, normal glucose tolerance is generally maintained (47).

Most research on the effects of diminished insulin secretory capacity has focused on metabolic changes which occur subsequent to the clinical onset of IDDM. This is partially due to the fact that during the pre-clinical phase of IDDM, individuals are asymptomatic and euglycemic, making it difficult to identify those who are progressing towards the overt disease. It is also difficult to predict those individuals who may be genetically susceptible to the disease, but have not yet been exposed to a precipitating ("trigger") event. Screening the general population, where ninety percent of the new cases of IDDM occur (108), would be an arduous task. Some attempts, however, have been made to study the "high risk" population (i.e. genetically matched siblings of IDDM's) as a means of providing insight into what changes in insulin secretion and insulin sensitivity may occur during the pre-clinical phase of IDDM (96, 97, 112). Additionally, insulin autoantibodies (IAA) (43, 150) and islet cell antibodies (ICA) (108) have been used as predictors of the disease. ICAs, which have been found in most patients at diagnosis of the disease, are the first and most reliable immunological predictive markers of IDDM (28, 29, 108). IAAs themselves have little predictive value for IDDM but, when found in association with high titer ICA's, their predictive capability is improved (150).

Human Investigations

As mentioned above, the ability of IV glucose to stimulate insulin secretion is diminished even before the overt onset of IDDM (46, 47, 125), yet the insulin response to non-glucose secretogogues and oral glucose is often preserved (54, 126). Ganda et al. ranked the insulin responses to a variety of secretagogues in four children in "early" stages of or remission from IDDM (54). At the time of experimentation, fasting plasma glucose values were within the normal range in these individuals. The rank order for the insulin response to a given secretogogue was determined as follows: IV arginine > IV glucagon > oral glucose > IV tolbutamide > IV glucose. IV glucose elicited virtually no insulin release, while the other stimuli prompted responses which ranged between 10% and 43% of the responses observed in normal control subjects. Results from this investigation indicate that the insulin response to IV glucose may be completely abolished even before total beta cell destruction has occurred and that during this time, the insulin response to other secretagogues is only partially lost.

Srikanta et al. provides additional data which demonstrates that the insulin response to IV glucose is the first secretagogue response to deteriorate (126). Furthermore, their data imply that this phenomenon may become evident during the pre-clinical phase of IDDM. They studied three islet cell antibody (ICA) positive, non-diabetic, first-degree relatives of IDDM subjects. Though these individuals displayed normal glucose tolerance, as determined via an oral glucose tolerance test, their insulin responses to IV glucose fell below the first percentile of the response displayed in normal controls. In contrast, there was a rapid release of insulin subsequent to administration of IV glucagon and there was a delayed, but apparent, secretory response following an oral glucose load. These three individuals did not, at the time of study, display clinical features of IDDM and it is not clear from the literature whether or not they progressed on to develop overt IDDM. However, results from this study as well as those from Ganda et al.'s investigation (54), imply that the insulin secretory response to IV glucose may be depressed or abolished during the pre-clinical phase of IDDM, while the responses to non-glucose secretogogues are preserved.

McCulloch et al. (96) studied the non-diabetic, genetically matched siblings of IDDMs in attempts to distinguish if subclinical beta cell loss is present in this population. The siblings were divided into two study groups, islet cell antibody (ICA) negative and ICA positive. Matched controls of families with no history of diabetes were also studied. This investigation demonstrated that the insulin secretory capacity was significantly lower in the ICA positive siblings than in the ICA negative siblings and controls. All the IDDM siblings, however, were below the controls when comparing the relationship between insulin secretion and sensitivity. Implicit in their results is not only an abnormality in insulin secretory function, but also in insulin action. The ICA negative siblings were not reported to have a decreased beta cell function when compared to controls, yet the relationship between insulin secretion and sensitivity was below the product of this relationship in the control subjects. Their results, therefore, suggest that the ICA positive siblings display impaired insulin secretion and possibly decreased insulin action, while the ICA negative siblings may exhibit insulin resistance, without a decrease in beta cell function. Further studies by the same group of investigators provide additional evidence for the presence of both insulin resistance and impaired beta cell function in non-diabetic genetically matched siblings of IDDM subjects (71).

In a recent publication (97) McCulloch and colleagues present a 5 year follow-up study on first-degree relatives of IDDM patients. This follow-up study was performed to determine if the presence of subclinical beta cell damage invariably leads to overt IDDM or if subclinical diabetes without progression to clinical symptoms is more common. The data from this study supports the conclusions from their previous investigations (71, 96), that subclinical beta cell dysfunction is present in non-diabetic first-degree relatives of IDDM patients. Furthermore, in this recent report it is concluded that in many cases the incidence of dysfunction is not progressive, i.e. does not lead to clinical IDDM. Since these subjects have only been followed for 5 years, the possibility cannot be ruled out that these individuals may eventually progress into a diabetic state.

Most recently, Palmer and McCulloch (108) reviewed the current knowledge concerning pre-clinical IDDM and the status of prediction as a step towards prevention of the disease. Based on their conclusion from the 5 year follow-up study (i.e. subclinical insulin deficiency does not necessarily lead to overt IDDM, (97)) and on evidence supporting the existence of IDDM remission, they hypothesize that the decline in beta cell function in IDDM is generally non-linear.

Prediction of Diabetes and the Linear Destruction Hypothesis

Results from several investigations performed in human subjects, which deal with the prediction of IDDM through the use of genetic and immunological markers, oppose Palmer and McCulloch's theory that beta cell function deteriorates in a non-linear fashion (43, 70, 125, 157). If the alternate hypothesis that beta cell loss is linear is true, defining the alterations in other metabolic parameters in relationship to the progression of beta cell dysfunction, could potentially lead to relatively simple methods for determining the time of diabetes onset in predisposed individuals. An animal model representing the "natural" progression of beta cell destruction would be ideal for exploring the relationships between a variety of metabolic parameters. Though such a model has not been previously developed, there has been some progress in human subject trials. Further investigation into the pathogenesis of IDDM is necessary before there can be routine prediction of the disease. Some investigators, however, have already proposed methods by which IDDM can be predicted, based upon the hypothesis that beta cell destruction is linear (70, 157).

Jackson and colleagues (70) developed the "dual parameter linear model for prediction of onset of type I diabetes". To develop the model they studied 33 islet cell antibody (ICA) positive relatives of patients with IDDM. The IVGTT was used to measure the first phase insulin response to glucose. This measurement was considered to reflect the degree of beta cell destruction. Anti-insulin autoantibodies (CIAA) were determined and were considered to be reflective of the rate of this destruction. A model equation was then developed using data from the first 14 of the 33 individuals who became diabetic. This equation was subsequently used to predict the number of years to commencement of clinical IDDM in the remainder of the subjects. Four out of 19 subjects exceeded their predicted time to disease onset, indicating that the parameters used in the formulation of this model were found to account for more than 75% of the variance in the time to IDDM development. The observed success of this model as a predictor of the time until IDDM onset, relies upon the assumption that beta cell destruction is linear during the preclinical phase of the disease.

Dotta et al. (43), who also supports the dual parameter model, reports that the concentration of insulin autoantibodies (IAA) in pre-diabetes correlates with the age at which diabetes develops and the rate at which islet cell antibody-positive individuals progress to diabetes. Thus, the data of Jackson et al. (70) and Dotta et al. (43) support the hypothesis that pre-clinical beta cell destruction occurs in a linear fashion.

The Joslin Study (157) was also performed to test the hypothesis that beta cell destruction in IDDM is linear. To investigate the pathogenesis of IDDM and to attempt prediction of the disease in susceptible relatives of IDDM patients, they employed three anti-islet antibody assays: an islet cell antibody assay, an insulin auto-antibody assay, and an assay to detect antibodies reacting with insulinoma cells. They report that the use of these three assays can detect more than 80% of pre-diabetic relatives of IDDM patients and nearly all individuals developing IDDM prior to age 5. Only the antibodies to insulin, however, appeared to reflect the age at which the disease develops. Though these individual antibody assays may aid the prediction of diabetes, the authors recognize the dual parameter model as being more predictive of the time of onset of overt IDDM. They believe that by using the present antibody assays and metabolic testing, it is now possible to identify first-degree relatives of IDDM patients who have a greater than 95% probability of developing IDDM within 3 years. Thus, the data presented in the Joslin study supports the hypothesis that once autoimmunity is activated, beta cell destruction during the pre-clinical phase of IDDM is linear.

Additional evidence to support the hypothesis that progressive beta cell dysfunction precedes clinical IDDM is found in a study performed by Srikanta et al. (125). Nine subjects who, for various reasons, were believed to be predisposed towards the development of IDDM were followed during the period preceding clinical symptoms up until onset of the overt disease. Elevations in fasting blood glucose and peak glucose during oral glucose tolerance tests were not seen until the year before onset of clinical diabetes. In contrast, a progressive loss of first phase insulin release in response to IV glucose was observed. The relationship between the linear loss of the insulin response to IV glucose and the number of years prior to overt diabetes, correlated well in all nine subjects (r=0.90). This observation provides additional support for the linear destruction theory.

It appears from the investigations performed in human subjects (43, 70, 125, 157), the linear destruction hypothesis of beta cell loss is gaining acceptance. An animal model of this phase of IDDM would be advantageous in studying the pathogenesis of the disease. Use of animals, however, to investigate pre-clinical beta cell dysfunction has been minimal due to the fact that conventional animal models of diabetes, such as those using diabetogenic doses of streptozotocin or alloxan, do not simulate the progression of beta cell destruction hypothesized to precede the onset of human IDDM. Additionally, spontaneously diabetic animal models, such as the BB/w rat, are difficult to use to study pre-diabetes. The incidence of spontaneous diabetes in the BB/w rat is approximately 30-50% (8, 88, 91) and unlike in human IDDM, the pre-diabetic phase in these animals is very short (91, 101).

BB/w Rat Model

Logothetopoulos et al. (91) studied the relationship between the initiation of beta cell destruction and the onset of overt diabetes in the BB/w rat. Since pronounced insulitis is present at the time of diabetes onset, they used it's presence as an indicator of beta cell destruction. Pancreatic biopsies were performed 2 to 53 days prior to the onset of overt diabetes, which was indicated by glucosuria. Sections of the biopsied specimens were examined via autoradiography to determine mononuclear infiltration. No infiltration was observed in any of the specimens obtained more than 18 days before the overt onset. Biopsies taken between 11 and 16 days prior to diabetes onset contained a few islets with mononuclear accumulations at the periphery or within the islet tissue. Most of the islets in these rats, however, appeared histologically normal. Sections from rats 9 or fewer days before onset of diabetes showed widespread mononuclear infiltrations of islet tissue. The closer the biopsy was obtained to the day of diabetes onset, the greater the extent of insulitis and loss of beta cells. This suggests that beta cell loss is progressive in the BB/w rat. Regardless of whether the loss is linear or not, results from this study, as well as those of Nakhooda et al.'s (101), suggest that the destruction is rapid during the pre-diabetic phase in the spontaneously diabetic BB/w rat.

Nakhooda et al. (101) used oral glucose tolerance tests (OGTT) to study BB/w rats prior to their developing diabetes. They defined the overt onset of the disease as the day on which glycosuria was first observed, which also coincided with the onset of hyperglycemia. In 3 out of 9 rats, results from the OGTTs were normal as close as 4 days prior to the onset of diabetes. The longest time span between the first abnormal OGTT and diabetes onset was 11 days. Their results demonstrated that the overt onset of diabetes in the BB/w rat is rapid, occurring over a period of only a few days.

Since the studies of Logothetopoulos et al. (91) and Nakhooda et al. (101) indicate that the pre-diabetic phase in the BB/w rat is rapid, in order to examine the pathogenesis of the disease within a given animal, it would be necessary to concentrate many experiments into a short time span (i.e. 16 days). The rat, however, has a small blood volume which would limit the number of experiments conducted within any one animal. Hence, due to the limitation of blood loss in such a small animal, the spontaneously diabetic BB/w rat would not be a viable model to longitudinally study the effects of progressive beta cell destruction on other metabolic parameters.

Summary

Studies performed in human subjects demonstrate that beta cell dysfunction, as well as insulin resistance, may exist in non-diabetic, genetically matched siblings of IDDM patients. Though it is not clear whether these impairments in the non-diabetic siblings are inherited or acquired or even if they always result in clinical IDDM, these abnormalities may characterize the pre-clinical phase of the disease. Presently, it appears that application of the dual parameter model provides the most hope for predicting the onset of IDDM, based on the hypothesis that beta cell loss occurs in a linear fashion. Through the promise of future exploration, a more thorough understanding of the pathogenesis of IDDM may lead to more simplified and common methods for diabetes prediction.

Insulin Sensitivity

In clinical IDDM, it is currently understood that a degree of impaired insulin sensitivity exists along with the severe insulin secretory dysfunction (17, 33, 42, 117, 154). There is no consensus, however, as to the mechanism which leads to this insulin resistance. Some data supports the theory that an impairment in insulin sensitivity is directly mediated via insulin deficiency (98), while other studies support the hypothesis that some metabolic derangement, such as hyperglycemia, is responsible (22, 52, 78, 100, 102, 118, 137).

A recent investigation by Hramiak and colleagues provides some insight into the importance of insulin sensitivity in the maintenance of glycemic control during subclinical insulin deficiency (unpublished observations). In newly diagnosed human IDDM subjects, it was demonstrated that non-insulin requiring remission from IDDM was more closely associated with a significant change in insulin sensitivity than a change in insulin secretory function. When compared to normal individuals, the newly diagnosed IDDM subjects displayed significant insulin resistance and insulin secretory dysfunction. Cyclosporin, an immunosuppressant, was administered to the IDDM subjects in attempts to induce remission. By 3 months, those who entered full non-insulin requiring remission had significantly improved insulin sensitivity which continued up until 6 months. By 9 months, however, insulin sensitivity had decreased to a level not significantly different from that prior to the remission period. Basal plasma C-peptide levels, which indicate insulin secretory function, increased significantly during the remission phase, but remained below the normal limits. In contrast to insulin sensitivity, the improved C-peptide level continued for at least 3 months after the relapse to diabetes. Thus, despite maintained improvement of basal C-peptide levels, relapse into IDDM occurred and was associated with the re-appearance of insulin resistance approximating that displayed prior to the remission. This data indicates that changes in insulin sensitivity are more closely associated with the development of and relapse from non-insulin requiring clinical remission in patients with newly diagnosed IDDM. Additionally, these results imply that a change in insulin sensitivity can occur independent of an alteration in beta cell function.

Normal Insulin Sensitivity

Demonstration of normal insulin sensitivity during beta cell dysfunction supports the hypothesis that changes in insulin sensitivity are not directly affected by changes in insulin secretory capacity.

A recent investigation performed by Kergoat et al. (78) provides evidence that normal insulin sensitivity can be present when beta cell function is severely impaired. Rats were studied 4 weeks after receiving 100 mg/kg streptozotocin neonatally. Though fasting hyperglycemia was not present, in comparison to controls, the streptozotocin treated rats were significantly hypoinsulinemic and insulin responses to IV glucose were significantly decreased. In contrast, clamp experiments demonstrated that the effect of insulin to suppress hepatic glucose production was normal in the drug treated animals, suggesting an absence of insulin resistance in the liver. Interestingly, glucose utilization was greater in the streptozotocin treated animals and in fact, measurement of whole body insulin-mediated glucose uptake was greater than that of control animals. This data, therefore, supports the theory that decreased insulin sensitivity is not induced by a primary lesion of the beta cell.

Further evidence from investigations performed by Finegood et al. (52) and Tobin et al. (135) indicates that a reduced insulin secretory response does not necessarily induce insulin resistance in islet-autotransplanted dogs. Despite a 71-91% reduction in insulin secretory function in these animals, normal fasting glucose and insulin levels were maintained. In addition, insulin sensitivity, as quantified via the MINMOD program applied to the FSIGT, was within the normal range. According to these results, it is possible for a significant insulin secretory deficit to be associated with normal insulin sensitivity, particularly when the level of insulin secreted is sufficient to maintain normal fasting glycemia.

Increased Insulin Sensitivity

Unger and Grundy (137) present the hypothesis that hyperglycemia is an inducer as well as a consequence of insulin resistance and offer the suggestion that this impairment reflects a post receptor defect, whereby depletion of intracellular glucose transport systems occurs. Support for their hypothesis is demonstrated in studies where reversal of insulin resistance occurs via normalization of plasma glucose concentrations, while beta cell function remains impaired.

Results from a study by Nankervis et al. (102) are in accordance with Unger and Grundy's (137) hypothesis. Nankervis and his colleagues studied the effects of insulin therapy on insulin action in individuals with newly diagnosed IDDM and demonstrated that the level of glycemia per se may directly affect insulin sensitivity. After one week of rigid insulin therapy, basal plasma glucose levels were significantly reduced. C-peptide levels were measured as an indicator of insulin secretory function and were found to be significantly lower in the untreated diabetics than control subjects, indicating an impairment in insulin secretory function. Subsequent to the insulin therapy, C-peptide concentrations rose, but this increase was not significant and continued to remain well below the levels found in the controls. Using glucose clamps, hepatic and peripheral insulin sensitivity were determined. In contrast to the maintained impairment in C-peptide, pre-existing hepatic insulin resistance was completely reversed by the insulin therapy. Peripheral glucose disposal, however, was only partially improved. The authors suggest that the unresolved peripheral insulin resistance was a post-receptor defect in glucose metabolism and that improved diabetic control would not reverse this defect. Of relevance, is the demonstration that despite continued insulin secretory dysfunction, as indicated by low C-peptide levels, improved glycemic control coincided with an improvement in insulin sensitivity in the insulin treated diabetics.

Rossetti et al. (118) uses a more direct method to determine if normalization of plasma glucose itself can reverse the insulin resistance observed in diabetes. They performed partial pancreatectomies in rats to induce moderate fasting hyperglycemia and post-meal glucose intolerance. The diabetic animals displayed a 27-29% reduction in insulin-mediated glucose disposal in comparison to control rats, as determined via the euglycemic hyperinsulinemic clamp. Phlorizin, an inhibitor of renal tubular glucose resorption, was then administered to the diabetic rats to normalize plasma glucose. Complete restoration of normal insulin sensitivity was associated with the normoglycemia. When phlorizin was discontinued, however, insulin resistance recurred to a similar degree as that prior to phlorizin treatment. These results indicate that hyperglycemia per se can induce insulin resistance. It is also suggested that the degree of insulin resistance is related to the severity of the hyperglycemia.

A more recent study using Phlorizin was performed by Blondel et al. (22). Streptozotocin diabetic rats were subsequently treated for 4 weeks with phlorizin to study what effect correction of hyperglycemia has on insulin sensitivity. Both liver and peripheral tissues displayed insulin resistance in the untreated diabetic rats. Fasting plasma glucose was 308 mg/dl, but was reduced to 142 mg/dl following phlorizin treatment. This improvement in glycemia was associated with normalization of glucose production by the liver and glucose utilization by the peripheral tissues. During this period of improved insulin action, beta cell function remained impaired to the same extent as prior to the phlorizin treatment. Results from this study are, therefore, in accordance with the proposal that hyperglycemia per se can lead to the development of insulin resistance.

Decreased Insulin Sensitivity

The evidence presented above supports the theory that a metabolic derangement, such as hyperglycemia, is probably responsible for the insulin resistance in IDDM; not beta cell deficiency itself. Data does exist, however, in opposition to this hypothesis. McCulloch et al. demonstrates that a reduction in insulin sensitivity can occur in the absence of hyperglycemia, as beta cell function deteriorates (98). Additionally, Raghu et al. (112) and Johnston et al. (71) provide data which indicate that insulin resistance may be present in non-diabetic, genetically matched siblings of IDDM subjects.

McCulloch and his colleagues studied the relationship between insulin secretion and insulin sensitivity during pre-clinical IDDM (98). These parameters were measured in baboons both in normal health and following treatment with low doses of streptozotocin (55 mg/kg for 3 days). In normal health, an inverse correlation was observed between insulin secretion and insulin sensitivity. One week subsequent to the streptozotocin administration, the acute insulin response to glucose was decreased by 62% and insulin sensitivity was decreased by 54%, yet fasting plasma glucose was normal. Since normoglycemia was maintained, the authors suggest that the observed insulin resistance is a direct effect of the impaired insulin secretory function, rather than a consequence of some other metabolic derangement. This conclusion opposes the hypothesis that insulin resistance occurs only after the onset of hyperglycemia.

Raghu et al. (112) also performed an investigation in attempts to clarify the mechanism behind the insulin resistance which develops in IDDM and when this impairment develops in regards to the disease process. They approached the question by studying insulin sensitivity in non-diabetic, genetically matched siblings of IDDM subjects. Twelve siblings of IDDM subjects were compared to age, sex, and weight matched controls from nondiabetic families. Fasting plasma glucose concentrations in the two groups were not significantly different. Using IVGTTs it was also determined that the insulin responses to IV glucose as well as the glucose tolerance values, were not different between the siblings and controls. In contrast, insulin sensitivity as calculated via the MINMOD computer program, was lower in the siblings of the IDDM subjects (siblings, 3.00 ± 0.24 versus controls, 5.31 ±0.57 x 10^4 [min^{-1}/(uU/ml)]; $p<0.01$).

At a later date, further investigations were performed in these same 12 non-diabetic genetically matched siblings, in order to determine whether their "normal" insulin secretory capacity was appropriate for their degree of insulin sensitivity (71). They hypothesized that the beta cell function tests performed in the siblings are inappropriate for direct comparison with those performed in other non-diabetic individuals. Thus, they adjusted the insulin secretion values for differences in insulin sensitivity. Re-analysis revealed that all measures of insulin secretion were significantly lower in the siblings and thus they conclude that both insulin resistance and impaired beta cell function exist in these individuals. This study, as well as the previous study (112), demonstrates that insulin resistance may be present in non-diabetic, genetically matched siblings of IDDM subjects. However, these results do not indicate whether this impairment is genetic or acquired.

Summary

As indicated by the literature, there is no consensus as to the mechanism which leads to the insulin resistance present in IDDM. Some data indicates that insulin sensitivity can remain within the normal limits while beta cell function is impaired, even to a severe extent. Other studies go further, suggesting that the ultimate initiator of insulin resistance in IDDM is hyperglycemia. Yet, other investigations imply that insulin sensitivity may be reduced prior to an elevation in plasma glucose and may possibly be influenced by beta cell dysfunction itself. To investigate the mechanism underlying the metabolic alteration in insulin sensitivity, an animal model of progressive beta cell dysfunction would be useful. By inducing a gradual decrease in insulin secretion, adequate time would be available to examine what consequences the deterioration in beta cell function may have on insulin sensitivity. Defining the relationship between subclinical insulin deficiency and insulin sensitivity may potentially benefit the prognosis of IDDM by contributing information needed to develop innovative intervention programs.

Glucose Effectiveness

The available information in the literature, regarding glucose effectiveness, is limited. There is some evidence which suggests that a reduction in glucose effectiveness is associated with subclinical insulin deficiency in both human subjects (50) and dogs (52, 135). Yet, other data indicates that diminished glucose effectiveness is not necessarily caused by insulin deficiency per se (18). Thus, the mechanism by which glucose effectiveness is decreased remains to be determined.

Reduced Glucose Effectiveness

A recent investigation performed in islet autotransplanted dogs provides support for the hypothesis that a reduction in glucose effectiveness develops following a period of chronic subclinical insulin deficiency (52, 135). Though insulin secretory function in these islet transplanted dogs was only 9-29% of control values, normal fasting plasma glucose, fasting plasma insulin, and insulin sensitivity were maintained. Glucose effectiveness, however, was significantly impaired. These results may indicate that the subclinical insulin deficiency somehow promotes a defect in glucose's ability to promote its own disposal, independent of an effect of glycemic level and insulin sensitivity.

Data from an investigation performed in human subjects (50) also supports the hypothesis that subclinical insulin deficiency may cause a reduction in glucose effectiveness. In this study, cyclosporin was administered to subjects with newly diagnosed IDDM in attempts to induce non-insulin requiring remission. In those patients who did enter full clinical remission, significant improvements were observed in insulin sensitivity, yet glucose effectiveness and insulin secretion remained chronically impaired. This study demonstrates that a defect in glucose action is present in IDDM. Furthermore, unless the impairment is due to previous hyperglycemia, the reduction in glucose effectiveness appears to be an effect of diminished insulin secretion.

Reduced Glucose Effectiveness Without Insulin Deficiency

If indeed subclinical insulin deficiency can cause a defect in the ability of glucose to enhance it's own disposal, it is not necessarily the "effector" in all cases. Data from a study by Bergman and colleagues (18) suggests that impaired glucose effectiveness may also be associated with insulin resistance, in the absence of impaired insulin secretion. Lean and obese subjects with normal basal plasma glucose, but with glucose intolerance, were found to have a significantly reduced glucose effectiveness when compared to lean and obese subjects with normal glucose tolerance. The etiology, however, of the glucose intolerance was entirely different in the lean and obese subjects, such that the lean subjects had normal insulin sensitivity, but impaired insulin secretion and the obese subjects had normal secretion, but decreased insulin sensitivity. Thus, a defect in the mechanism for insulin-independent glucose uptake may be associated with not only insulin deficiency, but also insulin resistance.

The presence of impaired glucose effectiveness in non-insulin dependent diabetes (NIDDM) also contributes to the notion that the defect is not only associated with insulin deficiency. Welch and colleagues (149) performed FSIGTs on subjects with NIDDM to measure total glucose uptake and insulin- (IMGU) and non-insulin-mediated glucose uptake (NIMGU). The insulin response to IV glucose was normal in 6 out of the 10 diabetic subjects. Not surprisingly, the individuals with NIDDM had markedly lower values of insulin sensitivity and total glucose uptake than controls. Though the diabetics displayed NIMGU values which were 40% lower than the controls, the contribution of NIMGU to the total glucose uptake was 77% versus 50% in control subjects. Thus, the impairment in NIMGU was less than that in IMGU. This study confirms the existence of impaired glucose effectiveness in NIDDM. However, whether or not the etiology of the defect is the same in both IDDM and NIDDM remains to be determined.

In opposition to the data of Welch and colleagues (149), Baron et al. (7) reports that non-insulin-mediated glucose uptake appears to function normally in NIDDM subjects. In fact, they report that individuals with NIDDM have basal rates of NIMGU that are approximately double the rate in normals. One significant difference between the two studies is that Welch et al. used the minimal-model to derive NIMGU, whereas Baron et al. measured NIMGU directly. The reason for the difference between the results from the two studies is unclear, however, variations in the degree of obesity, diabetic control or the suppression of endogenous insulin secretion in the different patient populations studied, may be contributing factors. In addition, the discrepancy may have been caused by an artifact in either of the methods of measurement. Perhaps when our knowledge of the etiology of impaired glucose action is improved, we will understand the reason for this conflict in data. Since little information is available on the mechanisms by which glucose action alters the regulation of glucose homeostasis in diabetes, we must also examine glucose action in other physiological states.

Glucose Effectiveness In Other Physiological States

A recent discovery in patients with cirrhosis provides insight into a possible site of a defect in glucose effectiveness (95). Insulin sensitivity and glucose effectiveness were determined via the MINMOD computer program in patients with cirrhosis and were found to be reduced by approximately 70 and 45%, respectively. The total amount of secreted insulin, however, was increased by approximately 90% during FSIGT experiments, when compared to controls. This data provides additional support for the idea that impaired glucose effectiveness can occur without insulin deficiency. Though the site and pathogenesis of the decreased glucose effectiveness cannot be definitively determined from this study, the authors propose that the defect is at the peripheral muscle tissue, rather than the liver. They suggest that since the liver does not take up glucose in great amounts and in cirrhosis, basal hepatic glucose production is normal or reduced, the defect may lie at the peripheral tissues. They also suggest that the muscle "wasting" which is characteristic in cirrhosis could somehow contribute to the decreased glucose effectiveness. To support their hypothesis, they show that in patients with cirrhosis there is a correlation between glucose effectiveness and urinary creatinine/height index (an indirect measure of muscle mass), which might suggest that the observed decrease in muscle mass is responsible for the majority of the impaired glucose effectiveness. Though their data implies a possible peripheral defect in glucose-dependent glucose utilization, the etiology of the defect remains to be determined. As well, the pathogenesis in cirrhosis may not be the same as in other disease states, such as diabetes.

Some populations in which glucose effectiveness was found to be normal are older men (35), women with histories of gestational diabetes (143), obese individuals with normal glucose tolerance (18), humans during norepinephrine infusion (94), and dogs during growth hormone infusion (1). The older (57-82 years old) men referred to above (35), displayed age-related glucose intolerance. In comparison to young (18-36 years old) men, the aged men exhibited both insulin resistance and a beta cell deficiency, but no differences were found in glucose effectiveness between the two groups. This implies that changes in insulin-independent glucose disappearance do not necessarily occur in age-related glucose intolerance and do not invariably accompany alterations in either beta cell function and/or insulin sensitivity. Further support for this can be found in women with histories of gestational diabetes (143), who have impairments in both insulin secretion and insulin action, but not glucose effectiveness.

Though it can only be speculated, it might be possible that a defect in non-insulin-mediated glucose disposal is independent of either changes in beta cell function or insulin action and instead, is indirectly affected by one or both of these parameters or is even related more directly to overall glucose tolerance. At any rate, further investigation is necessary to better understand the mechanisms by which glucose is able enhance its own disposal and the etiology of the defect in this parameter during the subclinical insulin deficiency associated with IDDM.

Summary

In this section I have summarized the current literature regarding insulin secretion, insulin sensitivity, and glucose effectiveness, during subclinical insulin deficiency. Recent data suggest that the progression of beta cell destruction in IDDM is linear. However, the pathogenesis of the disease and more specifically, the direct effects of subclinical insulin deficiency upon other parameters of glucose tolerance, are not thoroughly understood. In part, the lack of conclusive information in this area arises from the fact that diabetes is not one discrete disease, but is a heterogenous disease. In addition, dissimilarities between models of diabetes and the actual disease may contribute to the inconsistencies in the available data. Presently, it is accepted that insulin resistance, though not a primary defect, exists to some degree in overt IDDM. There is no consensus, however, with respect to the mechanism responsible for the impaired insulin action. Until recently, the role of glucose itself in promoting it's own disposal, has received little attention. Data obtained within the last three years suggest that glucose effectiveness is decreased during subclinical insulin deficiency. Future studies are necessary, however, to confirm this finding as well as to determine the mechanism by which this defect is induced. If we can improve our current understanding of the relationships between the defects in insulin secretion, insulin sensitivity, and glucose effectiveness during the pathogenesis of IDDM, progress may be made in the prediction, prevention, and intervention of IDDM.

E. STREPTOZOTOCIN

History

Streptozotocin, a naturally occurring antibiotic produced by Streptomyces achromogenes (138), was first isolated in 1959-1960 (65, 138). Then, in 1963 streptozotocin was discovered to be diabetogenic in rats and dogs (113). Later it was also found to have chemotherapeutic (48, 120) and carcinogenic (6, 155) properties. It wasn't until 1967, however, that the structure of streptozotocin was determined to be a methyl nitrosourea side chain linked to the C2 position of a D-glucose molecule (12, 66). Since these earlier studies, scientists have taken advantage of the diabetogenic properties of streptozotocin as a means to investigate the effects of insulin deficiency in laboratory animals.

Diabetogenic Action

The first investigation into the diabetogenic action of streptozotocin was performed by Rakieten et al. (113). In this original study, it was determined that the single IV dose of streptozotocin required to induce diabetes in both dogs and rats was 50 mg/kg. The minimum multiple dose regimen of streptozotocin capable of producing diabetes in dogs was found to be 15 mg/kg/day administered for 3 consecutive days. Doses of 10 mg/kg/day administered on 3 consecutive days were not diabetogenic. Though defining these limits would seem to provide control over the induction of diabetes, a specific dose that is diabetogenic in one animal, may not be in another animal of the same species, weight, and age (115). Thus, a disadvantage of using streptozotocin to experimentally induce diabetes, is that a specific dose does not consistently produce the same biochemical and morphological changes.

The basis for the unpredictable action of streptozotocin is not clear, but may be related to the mechanism by which beta cell destruction is induced by the drug. Though information concerning the mechanism of streptozotocin toxicity is limited, it is known that the damage to the pancreatic islet induced by a single, high dose of streptozotocin, involves different mechanisms than destruction via multiple, low doses of the drug (89, 103). Most studies in the literature have used a single, concentrated dose of streptozotocin to induce diabetes, while only a few have employed multiple dose regimens in which the beta cell toxin is administered over a period of 3-5 consecutive days (27, 59, 89, 113).

When a single diabetogenic dose of streptozotocin is administered, signs of direct drug-induced beta cell necrosis develop within 1-4 hours (30, 73). Frank necrosis is apparent by approximately 7-10 hours, as indicated by hypoglycemia resulting from the release of insulin which was contained in the damaged cells (73). Following this period of hypoglycemia, rapid onset of diabetes occurs, such that hyperglycemia is evident by 24 hours after the streptozotocin administration (73, 74). By one week, beta cells have degranulated, their organelles have been destroyed, and numerous macrophages have infiltrated the islets (127). There is virtually no inflammation, however, of islet lesions following a single diabetogenic dose of streptozotocin (30, 89).

In contrast to the effects of a single, high dose of streptozotocin, Like and Rossini (89) report that multiple subdiabetogenic injections of the drug (40 mg/kg administered on 3 consecutive days) induce a gradual increase in plasma glucose, with maximum values achieved one week or longer after the last drug injection. In the multiple dose regimens, beta cell necrosis is associated with mononuclear inflammatory cell infiltration in and around the pancreas, including large numbers of lymphocytes. Insulitis becomes detectable after 5-6 days following the last streptozotocin administration. Since development of insulitis is delayed in the multiple dose regimen and is virtually absent in the single dose regimen, it may be possible that multiple low doses of streptozotocin induce a cell-mediated immune reaction directed against the beta cells. Such an immune reaction itself could then precipitate further progression of beta cell destruction. This theory was initially proposed by Like and Rossini (89) based on their observation that progressive beta cell loss and increasing hyperglycemia occurred throughout a 10-25 day period following the final streptozotocin injection of a multiple dose regimen. During this period of delayed beta cell destruction, the drug would have long been cleared from the bloodstream (121) and it's cytotoxic action would have been complete (116). This indicates that the delayed beta cell loss is not a direct effect of streptozotocin, but supports the cell-mediated immune destruction hypothesis.

In attempts to better understand the diabetogenic mechanism of streptozotocin, Dulin et al. (44) studied the ability of a variety of compounds to block the drug's effect. 2-deoxyglucose, a glucose analog which competes with glucose for entry into the cell, was found to inhibit streptozotocin induced diabetes. It is suggested that the 2-deoxyglucose inhibits streptozotocin induced diabetes by "blocking" the entry of the toxin into the beta cell. Recalling that the structure of streptozotocin is a methyl nitrosourea side chain linked to the C2 position of a D-glucose molecule (12, 66), it may be possible that the glucose moiety of the toxin specifically directs it to its target, the beta cell.

Ganda et al. (53) also investigated the effects of several agents to inhibit the beta cell toxicity of streptozotocin. In these studies, D-glucose and D-mannose provided no protection from the drug's toxic effects. In contrast, almost complete protection was obtained when 3-O-methyl-D-glucose, a nonmetabolizable glucose analogue, was infused up to 5 minutes prior to streptozotocin administration. When 3-O-methyl-D-glucose was infused up to 30 seconds after streptozotocin administration, the protective effect was still present, but was significantly less than when infused prior to the streptozotocin. When the 3-O-methyl-D-glucose was infused 2 minutes following the streptozotocin, the protective effect was absent. Similar time-dependent protection was observed when 2-deoxyglucose was administered. They postulate that the effects of 3-O-methyl-D-glucose and 2-deoxyglucose are at the cell surface and that it is unlikely that intracellular events are responsible for streptozotocin's cytotoxic effect. This data maintains the idea that the glucose moiety of the streptozotocin molecule is a key element in the induction of streptozotocin diabetes.

Though the exact mechanism by which streptozotocin selectivity interacts with the pancreatic beta cell is not yet completely understood, studies by LeDoux and Wilson (83) and LeDoux et al. (84) contribute further insights into the drug's action which are consistent with previous findings. These studies support the hypothesis that it is the glucose moiety of streptozotocin which facilitates it's transport into the beta cells and which is important for the selectivity of it's action. Their results indicate that a defect in the glucose monitoring or glucose transport mechanisms which may occur after the first dose of the drug in a multiple dose regimen, may lead to a decreased uptake of the drug during subsequent dose administrations. This seems logical since, cells that lose their responsiveness to glucose should also lose their sensitivity to the glucose moiety of streptozotocin. An additional implication from these studies is that DNA damage ensues once streptozotocin enters the beta cell. LeDoux and colleagues believe that the streptozotocin decomposes to form carbonium ions which alkylate specific sites on DNA bases; these lesions are enzymatically removed, ultimately resulting in breaks in the DNA strands. This idea contradicts Ganda et al's (53) proposal that intracellular events are not responsible for streptozotocin toxicity.

Another hypothesis for the action of streptozotocin has been proposed by Wilson and colleagues (151). They suggest that upon entering the beta cell, streptozotocin not only alkylates DNA, but also alkylates key components necessary for the generation of ATP. If this theory is correct, this implies that at least some of the streptozotocin induced damage to the beta cell occurs past the glucose recognition site. Though this hypothesis has not yet been proven and the mechanisms by which streptozotocin induces diabetes are not completely elucidated, much progress has been made with regards to this query (152).

It remains unclear why, if the glucose moiety of the streptozotocin molecule is responsible for the entry of the toxin into the beta cell, other tissues which possess glucose transporters are not affected to the same extent as the beta cell. The specific characteristics of the glucose transporter isoform present on the beta cell, GLUT 2 (11), may be involved in directing the toxin specifically to the pancreatic islet. Though expression of the GLUT 2 transporter is found to be highest in the pancreas (130), it is also present in the liver, kidney, and small intestine (11). This is consistent with the toxic effects of streptozotocin; i.e. toxicity is greatest in the pancreas, however, it does appear that the drug has some effect on the kidney and liver (5, 6, 73, 86). As we better understand the diabetogenic effects of streptozotocin on the beta cell, we may also gain further insight into it's possible actions on other tissue structures.

Toxicity

Though streptozotocin treatment is used frequently in diabetes research as a method to induce beta cell dysfunction, the direct and indirect effects of the drug remain to be well defined. In an early study performed in streptozotocin treated rats, kidney and liver abnormalities were noted (73). These changes, however, were believed to be induced by the hyperglycemia secondary to the streptozotocin, and not due to the drug itself. Some evidence since then supports the possibility that streptozotocin alters the cytoarchitecture of tissues other than the beta cells of the pancreatic islets (5, 6, 86, 127). Tissues commonly believed to be affected by streptozotocin include the kidney tubules, hepatocytes, and lymphoid tissue. As well, a variety of host processes have been shown to be altered, including immunosuppression, renal function, lipogenesis, liver enzyme function, and adrenal steroid metabolism (3).

In one study (86), the toxicologic effects of single doses of streptozotocin were studied in six pairs of dogs and multiple doses (5 consecutive days) in five other pairs of dogs. It was demonstrated that single doses of 25, 12.5, and 6.3 mg/kg induced changes in lymphoid tissue, proximal convoluted renal tubules, hepatocytes and pancreatic islets. With repeated doses of 6.3 and 3.1 mg/kg, histologic lesions were also present in lymphoid tissue, renal tubular cells, hepatocytes, and the beta cells. At higher, multiple doses of streptozotocin additional damage was indicated by thymic hypoplasia, congestion of cecal mucosa, hemorrhages in the adrenal cortex, urinary bladder mucosa, and mesenteric lymph node, and necrosis of fat in the peripancreatic tissue. Overall, multiple dose regimens were less toxic than single dose regimens in terms of the total amount of the drug received. The important implication from this data is that the effect of streptozotocin is not limited to the pancreatic beta cell. Caution must be taken, however, since there has not yet been a clear delineation of the primary and secondary effects of streptozotocin toxicity.

In a review article, Rerup (116) recognizes that structures other than islet beta cells are affected by streptozotocin, though it has not been determined whether these changes directly emanate from the action of the drug itself. Rerup proposes that the diabetogenic action of streptozotocin occurs at the beta cell and that direct toxic effects are complete within a very short time, such that any histological and biochemical changes observed after approximately 15 minutes of IV drug administration are secondary effects of streptozotocin toxicity. This implies that in order to determine whether chronic insulin deficiency or streptozotocin per se causes the observed damage to other structures, tissues would need to be harvested almost immediately following drug administration. If streptozotocin is discovered to alter other tissues involved in glucose metabolism, it's usefulness in diabetes research may be limited. Hence, this possibility should be examined more closely.

Distribution

By studying the distribution of streptozotocin subsequent to its administration, additional insight may be gained with regards to it's toxicity. Ryo et al. (119) studied the distribution of ^{14}C- and ^{3}H-streptozotocin in dogs. Animals were killed 1,2,3,4,6 or 24 hours following an IV injection of labeled streptozotocin and blood was sampled from the heart chamber and tissues from various organs were assayed for radioactivity. The liver and kidney were found to have the greatest concentration of radiolabel at all time intervals. Surprisingly, they found no concentration of radiolabeled streptozotocin in the pancreas. However, the whole pancreas was used, instead of just using the islets. They hypothesize that even if radioactive streptozotocin were heavily accumulated in the islet tissue, it might not be sufficient to be detected on an assay of the whole pancreas. What this study does indicate is that the streptozotocin is being directed to the liver and kidney, where it may or may not have a direct toxic effect upon these organ tissues.

A similar study to Ryo et al.'s (119) was performed by Tjalve et al. (132). Mice were injected with ^{14}C-Streptozotocin (approximately 8.5 mg/kg) and subsequently killed at 5 minutes, 30 minutes, 1 hour, 4 hours, or 24 hours post drug administration. Autoradiography indicated that the greatest concentration of radiolabeled streptozotocin throughout the entire observation period, was in the pancreatic islets. At 5 minutes, 30 minutes, and 1 hour, the levels of radioactivity in the liver, kidney, urine, and gastrointestinal contents were high, but not exceeding the pancreatic islets. At 24 hours, the only tissues displaying radioactivity were the islets, liver, kidneys, salivary glands, Harder's gland, bone marrow, gastrointestinal mucosa, and the red pulp of the spleen. The urine and intestinal contents also showed radioactivity at 24 hours following the radiolabeled streptozotocin injection.

Results from both of these distribution studies (119, 132) demonstrate that streptozotocin is not only directed to the pancreatic islets, but is directed to other tissues as well. This is consistent with the hypothesis that streptozotocin toxicity affects a multiple number of sites. However, this does not necessarily mean that it's presence invariably leads to a direct toxic effect in these tissues. The relationship between the distribution of streptozotocin and it's direct toxic effects, is unknown at the present time.

Effects on Insulin Sensitivity

One question that remains to be determined is: does streptozotocin itself induce insulin resistance? Since this beta cell toxin is often used under experimental conditions to induce primary insulin deficiency (21, 80, 81, 87), this possibility should be evaluated more closely. Though there has not been a study performed to solely investigate this possibility, observations from studies employing streptozotocin provide some insight into this query. Data implying that insulin resistance appears to be directly related to insulin deficiency, originates from animal studies in which streptozotocin was administered to induce diabetes. Studies not employing streptozotocin generally do not arrive at this same conclusion. Instead they seem to indicate that an impairment in insulin sensitivity is not a direct consequence of beta cell dysfunction. The basis for these conflicting results could be related to an effect of streptozotocin itself to promote insulin resistance, independent of it's effect on the beta cell.

One study which demonstrates a direct relationship between streptozotocin induced insulin deficiency and insulin resistance was described in a previous section of this thesis (98). In brief, it was demonstrated that in streptozotocin treated baboons, the decrease in insulin secretory function associated with the streptozotocin administration, was correlated with a decline in insulin sensitivity, while fasting plasma glucose remained within normal limits. Though this one study by itself does not prove anything with regards to the capability of streptozotocin to promote insulin resistance, taken along with data from other studies, it may provide a basis for this hypothesis.

Conclusions from a study by Bevilacqua et al. (20), performed in streptozotocin treated animals, support the theory that insulin resistance can evolve from a primary lesion of the beta cell. They studied beta cell destruction in dogs, induced via 30 mg/kg streptozotocin. By 6 weeks following streptozotocin treatment, insulin secretory function had decreased by 75% and glucose clearance was significantly reduced from pre-treatment values. In this study, the degree of observed insulin resistance was directly correlated with the reduction in insulin secretion. They concluded that experimentally induced insulin deficiency leads to and is directly related to the development of insulin resistance. Though the possibility was considered that the observed peripheral insulin resistance may have been directly induced by streptozotocin, they feel this is not likely. Streptozotocin toxicity, though, as well as an effect of significantly elevated fasting plasma glucose, cannot be ruled out from the data presented in this study.

More apparent evidence that streptozotocin may cause a degree of insulin resistance is found in an investigation by Tsuji et al. (136). Two groups of rats were treated with 90 mg/kg streptozotocin, one group at 1.5 and the other at 5 days of age. Fasting plasma glucose concentrations in the group receiving streptozotocin at 1.5 days of age were the same as those in a group of control rats. Significantly higher fasting plasma glucose levels were demonstrated in the group receiving streptozotocin at 5 days. Ten weeks later, the 1.5 day old streptozotocin treated rats displayed insulin responses which were not significantly different from controls, while the insulin response in the group who received streptozotocin at 5 days was significantly impaired. Not surprisingly, the 5 day old streptozotocin treated rats displayed a reduction in glucose tolerance. Additionally, the rats injected with streptozotocin at 1.5 days had a mild impairment in glucose tolerance. Since insulin secretion was the same in this group as in the control rats, the glucose intolerance must be due to some other defect. The authors remark that a decrease in insulin action at the periphery may contribute to this glucose intolerance, yet no interpretation is offered to explain the origin of the insulin resistance demonstrated in the 1.5 day old streptozotocin treated group. These results are compatible with the hypothesis that streptozotocin itself may directly affect insulin action.

Finally, a study performed by Bonner-Weir et al. (25) is of particular interest with regards to the possibility of streptozotocin toxicity at the peripheral tissues. In this case, 2-day-old rats were treated with 90 mg/kg streptozotocin, resulting in a peak hyperglycemia at 4 days of age. By 10 days, plasma glucose concentrations had returned to normal. However, by 6 weeks of age hyperglycemic levels resumed and persisted throughout the remainder of the study. The insulin secretory function in these animals decreased by only 25%, which by itself does not generally induce hyperglycemia. It appears then, that some other parameter of glucose tolerance was altered to promote the development of hyperglycemia in these streptozotocin treated animals. One might speculate that an impairment in insulin sensitivity was partially responsible for the hyperglycemia. If insulin resistance was present, could it have been induced by some mechanism of streptozotocin toxicity?

Though there is not any concrete evidence that streptozotocin itself causes insulin resistance, some data seems to suggest that this possibility does exist. If this hypothesis proved to be true, more careful interpretation of studies employing streptozotocin would be necessary. Hence, it appears that further examination of streptozotocin's action is warranted.

II. RESEARCH PLAN

A. GLOBAL OBJECTIVE

To elucidate the metabolic alterations associated with progressive deterioration of insulin secretory function.

B. SPECIFIC AIMS

1) To establish an animal model of progressive insulin secretory dysfunction.
2) To determine the longitudinal relationship between a gradual decline in insulin secretory function and insulin sensitivity, glucose effectiveness, and glucose tolerance.
3) To determine the relationship between the acute insulin response to glucose (AIRg) from the exogenous insulin modified frequently sampled intravenous glucose tolerance test and the slope of glucose potentiation of the acute insulin response to arginine (AIRarg), over a wide range of beta cell function.

C. RATIONAL

Aim #1

In the past, diabetes research has focused on metabolic alterations subsequent to the onset of overt hyperglycemia. Development of an animal model of insulin deficiency which resembles the progression of beta cell dysfunction found in the pre-clinical phase of IDDM, would be valuable as a tool to investigate the effect of this gradual impairment on the other parameters of glucose tolerance. Hence, the first aim of this thesis research was to establish an animal model of progressive insulin secretory dysfunction.

Aim #2

In IDDM, it is not yet known if the decrease in insulin secretion per se leads to the observed decrease in insulin sensitivity. Nor is it known how or if alterations in insulin sensitivity, glucose effectiveness, or glucose tolerance occur with the decline in insulin secretion prior to the acute onset of IDDM. An understanding of the direct and indirect actions of insulin secretion may be important in the IDDM during the course of pre-clinical diabetes, remission from diabetes, or in the long term effect of islet/pancreas transplantation. Through increased knowledge of the metabolic alterations preceding diabetes, individualized intervention programs may be developed in attempts to prevent or delay the disease process. Thus, the second aim of this thesis was to determine the longitudinal relationship between a gradual decline in insulin secretory function and insulin sensitivity, glucose effectiveness, and glucose tolerance.

Aim #3

The ability to quantitate the degree of beta cell dysfunction, both during the pre-clinical and clinical stages of IDDM, is advantageous in the development of individualized treatment therapies. A variety of secretagogues, including glucose and arginine, have been utilized to induce an insulin response for the purpose of characterizing beta cell function. Opinions vary as to which secretagogue and which method of measuring insulin secretory function is best. The slope of potentiation has been advocated as being the most sensitive indicator of beta cell function (98). Therefore, if a high correlation exists between the AIRg from the FSIGT and the slope of potentiation over the spectrum of beta cell function, then the AIRg can be used as a reliable measure of insulin secretion. The benefits of validating the FSIGT AIRg as a measure of insulin secretion are 1) it allows determination of insulin sensitivity, glucose effectiveness, and glucose tolerance in addition to insulin secretion and 2) it is easier and costs less to perform than the slope of potentiation. Thus, the third aim of this thesis project was to determine the relationship between the AIRg from the FSIGT and the slope of glucose potentiation of the AIRarg, over a wide range of beta cell function in dogs.

III. EXPERIMENTAL DESIGN AND METHODS

A. ANIMALS

Six adult male mongrel dogs with body weights ranging from 24 to 36 kg were fed 600 - 800 grams of Tuffy's Dog Food Chunks (Tuffy's Division of Star-Kist Foods Inc., Perham, Minn.), once daily and provided free access to water. Animals were fasted 19 hours prior to streptozotocin administration and experimentation.

Dogs were housed in individual pens and were isolated in metabolic cages for three days following streptozotocin treatment. Isolation procedures were employed because it has been shown that the principal route of streptozotocin excretion is the urine (119) and at 24 hours post streptozotocin treatment, urine still contains small quantities of the drug (119, 132).

Prior to being accepted into the study, each dog was familiarized with the laboratory. Animals who adapted well to restraint in a Pavlov sling and percutaneous catheterization, were determined suitable for the study. All dogs were under direct supervision of a veterinarian and all experimental procedures were approved by the Health Sciences Animal Welfare Committee of the University of Alberta in accordance with the guidelines of the Canadian Council on Animal Care.

B. STREPTOZOTOCIN TREATMENT

Streptozotocin is unstable at room and refrigerator temperatures. In a saline or distilled water solution at room temperature and neutral pH, it decomposes within a few minutes (65, 116). Maximum stability of the compound occurs at low temperatures and in solution; a pH of 4.0 is optimal to prevent decomposition (65, 113). Thus, in the present study streptozotocin (Sigma Chemical Company, St. Louis, MO) was diluted under sterile conditions in a 0.051 M solution of citric acid/sodium acetate buffer at pH 4.08, refrigerated until use, and injected within 1 hour of mixing. A saphenous vein was cannulated via a 20 g intracatheter (Quikcath, Travenol, Deerfield IL) and 10 mg/kg body weight of streptozotocin in solution was infused. Animals were allowed to recover for 7 days following streptozotocin administration.

Streptozotocin was administered at 14 day intervals and was discontinued following 10 doses (n=4), the onset of fasting hyperglycemia (n=1), or death (n=1). In the initial dog, who became hyperglycemic following the sixth streptozotocin dose, drug treatment intervals ranged between 7 and 33 days (mean interval ± STD, 14 ± 10 days). Since results from this dog were congruent with those from the other study animals, this data is included in the results presented in this thesis.

C. EXPERIMENTAL PROTOCOLS

Prior to administering the initial streptozotocin dose, an insulin modified FSIGT and a slope of potentiation protocol were performed on each dog. Though it is recognized that it would have been ideal to also include a sham streptozotocin treated dog as an independent control, the pre-streptozotocin data obtained from each dog behaved as it's own control against which all subsequent data from that dog were compared. Due to the longitudinal nature of this "within subjects" study, pre-treatment data are considered to be adequate for control comparisons.

Following each streptozotocin administration, an insulin modified FSIGT and a slope of potentiation protocol were performed (8 and 10 days post streptozotocin treatment, respectively). Only FSIGT's were performed on the one animal with the varying streptozotocin treatment intervals. Thus, a total of 56 FSIGT and 49 slope of potentiation experiments were performed. Experiments were performed on conscious animals restrained in a Pavlov sling, which allowed them to stand or sit comfortably.

Insulin Modified Frequently Sampled Intravenous Glucose Tolerance Test

The following parameters were quantified from the insulin modified FSIGT: the acute insulin response to glucose (AIRg), the integrated insulin response to glucose (IIRg), glucose tolerance (K_G), insulin sensitivity (S_I), and glucose effectiveness (S_G). The standard FSIGT protocol requires an endogenous insulin response to glucose and tolbutamide challenges for the estimation of S_I (15). Since streptozotocin treated animals might not secrete sufficient amounts of insulin in response to tolbutamide to allow S_I determination, we replaced the tolbutamide injection with an insulin injection to provide sufficient circulating insulin levels (148). As discussed in the literature review, application of an exogenous insulin injection FSIGT protocol for S_I and S_G determination, using the minimal-model of glucose kinetics, has recently been validated by Finegood and colleagues, in both humans (50) and dogs (49).

For sampling and test substance administration, the saphenous vein was catheterized with an 18 g intracatheter connected to an extension line filled with heparinized saline (100 U/ml heparin). A heating pad was placed on the catheterized leg to promote blood flow, following which animals were allowed to rest quietly for 15 minutes prior to basal blood sampling. Four samples were drawn between -20 and 0 minutes for basal measurement of plasma glucose and insulin. Exogenous glucose (0.3 g/kg, 50% dextrose; Abbott, Montreal) and insulin (0.03 U/kg) (148) were injected at 0 and 20 minutes, respectively. Samples were drawn at 2, 3, 4, 6, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 40, 50, 60, 70, 80, 100, 120, 140, 160, and 180 minutes for plasma glucose and insulin determinations. Total blood loss was less than 150 ml per experiment.

Insulin for injection during a given experiment was prepared under sterile conditions the morning of that experiment (0.5 U/ml), using purified regular pork insulin (Eli Lilly, Indianapolis, IN) in normal saline and the dog's own plasma.

Slope of Glucose Potentiation

The slope of glucose potentiation protocol was used to determine the acute insulin response to arginine (AIRarg) at basal glucose, the AIRarg at hyperglycemia, and the slope of potentiation of the AIRarg (slope). Two veins (saphenous and/or cephalic) were catheterized with 18 g intracatheters. One vein was used for arginine administration and sampling and the other vein was used to infuse glucose. A heating pad was placed on the leg used for sampling, as was done in the FSIGT protocol. Basal sampling commenced at -20 minutes. At time 0 minutes a 5 gram bolus of arginine (250 $mg \cdot ml^{-1}$ as arginine hydrochloride; Sabex, Montreal QUE) was administered over 0.5 minutes. Samples were drawn at 2, 3, 4, 5, 7, 12, 20, 25, 30, and 35 minutes. At 40 minutes a variable rate continuous infusion of glucose (50% dextrose) was delivered via a peristaltic roller pump (Instech Laboratories, Horsham PA; Model 420-C). Plasma glucose was determined immediately after sampling via a Beckman II glucose analyzer (Beckman Instruments, Fullerton, CA). Subsequent adjustments to the glucose infusion were made in attempts to maintain a relatively constant blood glucose of 19.4 mM. Samples were drawn at 42, 44, 46, 48, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, and 130 minutes. At 135 minutes a second bolus of arginine (5 grams) was administered and frequent sampling commenced as following the first arginine pulse, with the final sample obtained at 165 minutes. Total blood loss was less than 200 ml per experiment.

D. MONITORING AND ASSESSMENT

Prior to an animal receiving its fifth streptozotocin dose, fasting blood glucose (FBG) was monitored every other day. Following the fifth dose, FBG was monitored daily. The Glucometer II / Glucostix system (Diagnostics Division, Miles Canada Inc., Etobicoke, Ontario) was used for immediate interpretation of FBG and for more accurate analysis, an additional plasma sample was collected for future assay.

Body weight, hemoglobin (Hgb), hematocrit (Hct), white and red blood cell counts (WBC, RBC), blood urea nitrogen (BUN), and creatinine levels were monitored weekly to evaluate each animal's general health. Abnormal values for any of these lab parameters can be indicative of a wide variety of clinical problems. In the present study, however, the most probable abnormalities which may contribute to alterations in these parameters include: 1) changes in blood volume (i.e. secondary to blood loss, overhydration, or dehydration) which can alter Hgb, Hct, and RBC counts, 2) acute infections and uncontrolled diabetes which can lead to abnormal WBC counts, and 3) kidney disease (i.e. glomerular nephritis) and acute or chronic renal failure which can elevate BUN and creatinine levels.

Assays for Hgb, Hct, WBC and RBC counts, BUN, and creatinine were performed by the Surgical Medical Research Institute at the University of Alberta. The Dual Diluter III (Coulter Electronics Ltd., Luton England) and the Coulter Counter M430 (Coulter Electronics, Hialeah Florida) were used for Hgb, Hct, WBC, and RBC determinations. BUN and creatinine determinations were made using the Multistat III Centrifugal Loader and Micro Centrifugal Analyzer (Multistat III Instrumentation Laboratory Inc., Micro Chemical Division, Spokane, WA).

Urinalysis was performed bi-weekly to check for glucose (Chemstrip 9, Boehringer Mannheim Canada Ltd., Mannheim, Germany) and casts (Kova System Stain, ICL Scientific, Garden Grove, CA). Glucosuria can occur for a wide variety of reasons, including diabetes and kidney damage associated with significant tubular lesions. Tubular casts, also indicative of kidney damage, are composed primarily of a mucoprotein secreted by the epithelial cells that line the renal tubules (106). They are cylindrical in shape and are literally a "cast" of the tubular lumen. Detection of significant numbers of casts in the urine indicates that an active pathologic process is occurring in the tubules. Large quantities of casts indicate active generalized renal disease, which is usually acute, while small quantities may occur in acute or chronic renal disease.

Upon completion of the study, animals were euthanized via an overdose of sodium pentobarbital (Euthanal). Necropsies were performed on five of the six dogs to evaluate morphological and pathological changes which may have occurred as a result of the insulin deficiency or streptozotocin toxicity. Tissue sections from the kidney, spleen, liver, and pancreas were obtained for histologic examination. Necropsies were performed by Dr. P.N. Nation at the Alberta Agriculture Animal Health Division, Veterinary Laboratory.

E. SAMPLE HANDLING AND ANALYSIS

Blood for plasma insulin and glucose determinations was put into tubes containing heparin (150 U/tube) and NaF (2 mg/tube). Blood for glucagon was aliquoted into green top vacutainer tubes (45 usp Units sodium heparin; Becton Dickinson and Company, Rutherford, NJ) with 1000 KIU trasylol added (Miles Laboratories, Etobicoke, Ontario). All samples were immediately placed on ice, centrifuged (4000 rpm; 12 min; 4^{o}C) within 30 minutes of collection, and the plasma was separated and frozen (-20^{o}C) for future assay.

Plasma glucose concentrations were measured in duplicate by the glucose oxidase technique using a Beckman II Glucose Analyzer (Beckman Instruments, Fullerton, CA). Plasma insulin was determined by competitive binding radioimmunoassay using a two antibody system and human insulin standards (Pharmacia, Insulin RIA 100, Uppsala Sweden). Plasma glucagon was analyzed via a double antibody technique using 04A pancreatic glucagon antiserum (Univ. Texas; Dallas) and I^{125} glucagon (CNR 120, Cambridge Medical Diagnostics). Plasma insulin and glucagon assays were performed by Eva Dolinski in the Muttart Diabetes Research and Training Center.

F. CALCULATIONS

Insulin Modified Frequently Sampled Intravenous Glucose Tolerance Test

For statistical analysis, measurements of fasting plasma glucose and insulin were derived by averaging the values from the 4 samples prior to the glucose injection in the FSIGT. S_I and S_G and their respective fractional standard deviations were calculated with the MINMOD computer program (107) (version NUDEMM1, copyright 1986, R.N. Bergman). K_G was calculated as the slope of the linear regression of the natural logarithm of the plasma glucose versus time, prior to the insulin injection (minutes 8-19).

The integrated insulin response to glucose was calculated as the area under the insulin concentration curve, above basal, between 0 and 19 minutes. The AIRg was calculated as the average insulin concentration in the 2 - 5 minutes following the glucose injection minus the average insulin concentration in the 20 minutes prior to the glucose injection.

Slope of Glucose Potentiation

The AIRarg at basal, the AIRarg at hyperglycemia, and the slope were all derived from the slope of potentiation protocol. The AIRarg was calculated as the mean insulin concentration in the 2 - 5 minutes post arginine injection minus the mean insulin concentration in the 15 minutes prior to the arginine injection. The average plasma glucose concentration in the 15 minutes prior to the arginine injection was used as the pre-stimulus steady state concentration.

The AIRarg's were plotted against their respective steady state glucose concentrations (basal and hyperglycemia) and the slope of the line formed by the two points was determined. This measurement is the slope of potentiation of the AIRarg. Since there were only two points used to formulate the line, the change in Y (AIRarg) divided by the change in X (glucose concentration) was used to determine the slope. Though it would have been ideal to have additional data points with which to determine a regression line, due to the humane constraints of blood loss in the present experimental design, further blood samples were not collected. In support of using two data points to calculate the slope of potentiation, it has been previouly demonstrated that the linearity of the relationship between the plasma glucose concentration and the AIRarg is maintained at glycemic levels lower than 19.4 mM (144). In addition, unpublished observations from our laboratory demonstrate that in islet autografted dogs a linear relationship exists between the glycemic level and the AIRarg up to a plasma glucose concentration of 27.8 mM. Since the AIRarg at hyperglycemia was measured at approximately 19.4 mM in the present study, the relationship between the plasma glucose and the AIRarg should have been within the linear range.

The integrated glucagon response to arginine was calculated as the area under the glucagon concentration curve, above steady-state, during the 12 minutes immediately following the arginine injection. Steady state glucagon was calculated via averaging the glucagon concentration in the 10 minutes prior to arginine stimulation. Glucagon integrations were performed at euglycemic and at hyperglycemic levels. For statistical purposes, the steady state glucagon values obtained at basal glycemia were also used to represent fasting plasma glucagon.

G. STATISTICS

The Statistical Analysis System (SAS; ver. 6; Cary, NC) was used for most statistical calculations. The general linear models (GLM) procedure was used to test for repeated measures analysis of variance (ANOVA) differences for each parameter and monitored variable. Results at each streptozotocin dose (level) were contrasted with the pre-streptozotocin result and each level was contrasted to the successive level. Pearson product moment correlations were performed to determine relationships between streptozotocin dose, insulin secretion, S_I, S_G, K_G, fasting plasma insulin, and fasting plasma glucose, as well as correlations between the various measures of insulin secretion. Correlations by dog and by dose were determined when necessary to separate between and within dog effects.

The stepwise linear regression procedure in SAS was used to assess the contribution of various parameters in determining glucose tolerance. K_G was entered as the dependent variable. Independent variables tested included fasting plasma glucose and insulin, the integrated insulin response to glucose, S_I, and S_G. The significance level for entry into the model, as well as for staying in the model, was $p<0.15$.

Minitab (Minitab Data Analysis Software, Minitab Inc., State College, PA; Release 82.1) was used to perform linear regression and a lack of fit test (LOF) to determine if a linear relationship exits between the streptozotocin dose and insulin secretion. Log transformed values for insulin secretion were also tested for LOF with cumulative streptozotocin dose.

All comparisons were considered statistically significant at $p \leq 0.05$. All values are reported as mean ± standard deviation, unless otherwise noted.

IV. RESULTS

A. GENERAL RESULTS

Animals

Of the six dogs studied, 4 received 10 streptozotocin doses without developing fasting hyperglycemia, 1 animal developed fasting hyperglycemia (7.5 - 12.4 mM) following 6 doses, and 1 animal died of severe glomerulonephritis following the 5th streptozotocin dose.

Monitored Variables

Table 1 shows the mean animal weights at each streptozotocin dose level. Repeated measures analysis of variance revealed no significant weight changes when the weight at each streptozotocin level was contrasted to the pre-streptozotocin weight ($p>0.05$).

Table 2 displays the mean values for each lab parameter at each streptozotocin dose level. Due to limited data from the two animals who did not complete all 10 streptozotocin doses, statistical calculations on the lab parameters include only the four animals who received 10 streptozotocin treatments. Hemoglobin and hematocrit slightly but significantly declined throughout the study period ($p=0.0006$ and $p=0.02$ respectively). Although the decline was significant, hematocrit remained within normal limits throughout the study, beginning with a pre-streptozotocin mean value of 43 ± 2% and ending the study with a mean value of 39 ± 2% (normal range, 35-53%).

Table IV-1. Individual and mean dog weights at each streptozotocin dose level.

Dog I.D.	Cumulative Streptozotocin Dose (mg/kg) 0	10	20	30	40	50	60	70	80	90	100
J551	32.9	32.2	31.1	31.1	30.5	29.2	28.8	-	-	-	-
J587	32.5	31.1	30.5	30.2	29.7	-	-	-	-	-	-
K331	28.6	28.2	28.7	29.1	28.9	28.7	28.5	28.3	28.0	28.4	28.5
K430	28.1	28.6	28.6	28.7	28.4	28.2	28.4	27.8	27.9	28.4	28.2
L26	26.9	26.1	26.0	26.1	25.6	25.6	25.9	25.5	25.7	25.2	24.1
L57	34.6	35.4	35.7	35.7	35.2	34.7	34.6	34.9	34.6	34.2	32.8
mean body wt (kg)	30.6 ±3.1 (n=6)	30.3 ±3.3 (n=6)	30.1 ±3.3 (n=6)	30.2 ±3.2 (n=6)	29.7 ±3.2 (n=6)	29.3 ±3.3 (n=5)	29.3 ±3.2 (n=5)	29.1 ±4.0 (n=4)	29.1 ±3.8 (n=4)	29.0 ±3.7 (n=4)	28.4 ±3.6 (n=4)

The upper panel displays individual dog weights (kg) at each streptozotocin dose level. Values in the lower panel represent means ± STD at each streptozotocin dose level. Repeated measures analysis of variance reveals no significant weight changes when the weight at the pre-streptozotocin dose level is contrasted to weights at subsequent dose levels ($p>0.1$).

Table IV-2. Mean laboratory test values at each streptozotocin dose level.

	Cumulative Streptozotocin Dose (mg/kg)										
Parameter	0	10	20	30	40	50	60	70	80	90	100
Hct (%)	43 ±2	45 ±1	45* ±.9	46 ±4	44 ±4	43 ±5	43 ±4	42 ±5	39* ±3	41 ±5	39* ±2
Hgb (mM/L)	9.29 ±.52	9.31 ±.42	9.47 ±.47	9.60 ±1.03	9.15 ±.98	9.11 ±1.37	8.91 ±1.01	8.50 ±1.20	8.06 ±.89	8.30 ±1.33	7.77 ±.62
WBC (10^9/L)	9.6 ±.9	9.2 ±2.2	9.8 ±2.2	9.9 ±2.6	9.7 ±2.1	9.9 ±2.1	9.6 ±1.9	9.1 ±2.0	9.5 ±2.3	8.5 ±1.9	7.8 ±1.3
RBC (10^{12}/L)	6.1 ±.4	6.2 ±.2	6.3 ±.3	6.3 ±.5	6.2 ±.5	6.5 ±.4	6.5* ±.4	6.4 ±.3	6.3 ±.2	6.6 ±.3	6.5 ±.3
BUN (mM/L)	4.9 ±1.3	5.4 ±.8	5.5 ±1.1	5.2 ±1.5	4.6 ±1.0	4.2 ±.8	4.6 ±.4	4.2 ±.2	3.7 ±.5	4.4 ±1.2	3.9 ±.7
Creatinine (uM/L)	96 ±13	103 ±10	110 ±30	108 ±23	112 ±16	113 ±19	112 ±27	111 ±31	86 ±24	100 ±13	88 ±32

Values represent means ± STD at each streptozotocin dose level ($n=4$). Significant differences from the pre-streptozotocin dose level are indicated with a * ($p<0.03$).

Hemoglobin only dropped below the normal range following the final streptozotocin dose, declining from a pre-streptozotocin value of 9.29 ± 0.52 mM/L and reaching a nadir of 7.77 ± 0.62 mM/L subsequent to the 10th streptozotocin dose (normal range, 7.88 - 10.12 mM/L). There were no significant changes in white blood cell counts (9.3 ± 2.1 $\times 10^9$/L, p=0.28), red blood cell counts (6.4 ± 0.4 $\times 10^{12}$/L, p=0.40), blood urea nitrogen (4.6 ± 1.0 mM/L, p=0.20), or creatinine (103.5 ± 21.9 uM/L, p=0.30).

Fasting Plasma Glucose, Insulin, and Glucagon

Univariate testing for within subject effects revealed no significant changes in fasting plasma glucose (FPG) throughout the study period (p=0.71; pre-streptozotocin FPG ± STD, 5.06 ± 0.27 mM; post 100 mg/kg cumulative streptozotocin FPG ± STD, 5.18 ± 0.91 mM, figure 1). When FPG at each streptozotocin level was compared to pre-streptozotocin values, FPG decreased following the 9th streptozotocin dose (p=0.005), but resumed values not significant from basal following the 10th and final streptozotocin dose (p=0.9). Fasting plasma insulin (FPI) and fasting plasma glucagon levels did not change significantly throughout the study (p=0.15 and p=0.07, respectively; pre-streptozotocin FPI ± STD, 41.33 ± 25.07 pM; post 100 mg/kg cumulative streptozotocin FPI ± STD, 22.25 ± 5.32 pM; pre-streptozotocin fasting plasma glucagon ± STD, 117.9 ± 13.86 ng/L; post 100 mg/kg cumulative streptozotocin fasting plasma glucagon ± STD, 147.37 ± 42.91 ng/L; figure 1).

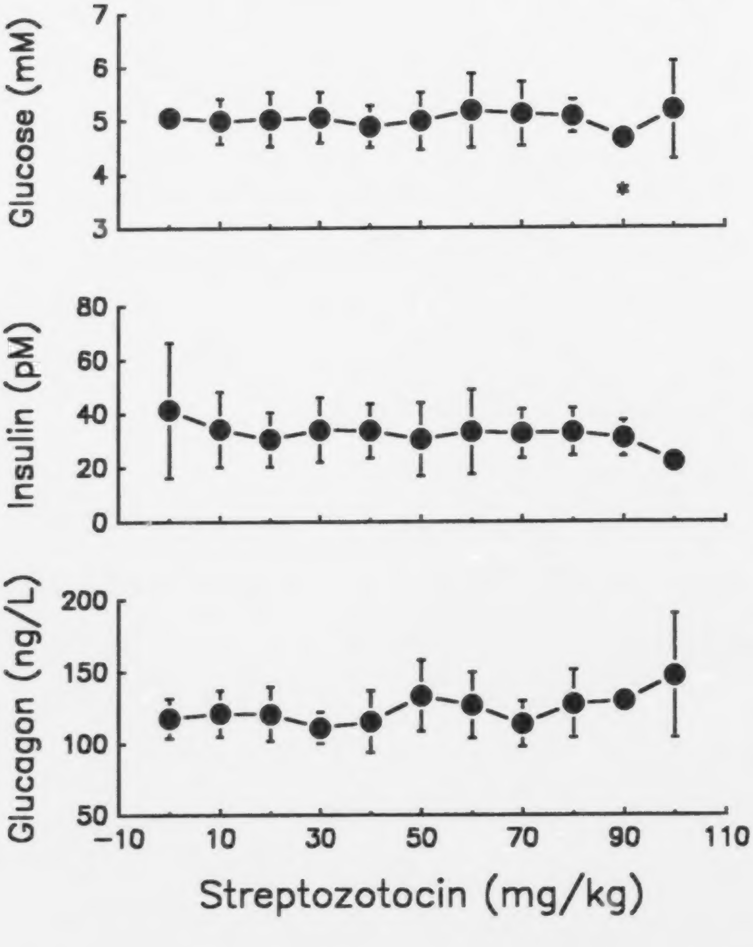


Figure IV-1. Fasting plasma glucose, insulin, and glucagon as a function of cumulative streptozotocin dose.

The * indicates a significant difference from the pre-streptozotocin value (p=0.005). Values are means ± STD.

Postmortem

Under ideal circumstances, it would have been beneficial to quantitate the histopathology results in order to determine if any correlations exist between tissue destruction and the parameters of glucose tolerance. Due to the limitations of this study, however, only qualitative observations will be reported. All postmortem data was collected by Dr. P.N. Nation and not the author.

The one animal who died following the fifth streptozotocin dose was diagnosed as having severe glomerulonephritis. Approximately 1/3 of each kidney was affected with severe multifocal pitting and dark discoloration of the surfaces. This was believed to be compatible with subacute to chronic multifocal infarction. Additionally, scaring was observed, which may have been indicative of previous episodes of infarction and moderate acute to subacute nephrosis. The glomerulonephritis was nonspecific but was of a type common in dogs and thought to be usually immune mediated. The more acute nephrosis was also nonspecific but was compatible with streptozotocin toxicity.

Histopathology results common to all dogs, indicated destruction had occurred in the pancreatic islets of Langerhans. Surviving islets exhibited disorganized hypocellular structures and the cells within the islets were small in size. These remaining islet cells had scant amounts of cytoplasm and considerable variation in nuclear size. Occasional collapsed islet remnants were also visible.

Tubular nephrosis was commonly observed in the kidneys. This abnormality was characterized by a decrease in the number of renal tubular epithelial cells, an increased size of the nucleus in these cells, and variable orientation of the nucleus. Though not found in the other animals, the dog that died after 5 streptozotocin doses was found to have glucose (30 mM/L) in the urine.

Another general observation was made in the spleen, where there was extensive depletion of lymphocytes. In the liver, however, alterations were not consistent throughout all animals. In two dogs, acute congestion of the liver was noted; in another, degeneration of hepatocytes and variation in their nuclear size was observed; and in two dogs, lesions interfering with bile excretion were found. In one of these animals there was specific damage to the biliary tree such that there was leakage of bile, as evidenced by the presence of bile containing macrophages in the connective tissue of the liver. The Kupffer cells (cells in the liver which filter bacteria and other small, foreign proteins out of the blood) were also found to contain bile pigment. Additionally, in this animal the hepatocytes were small and the cytoplasm of these cells was vacuolated.

B. INSULIN MODIFIED FREQUENTLY SAMPLED INTRAVENOUS GLUCOSE TOLERANCE TEST

Figure 2 depicts the mean FSIGT time courses for plasma insulin and glucose prior to streptozotocin treatment and subsequent to the final treatment, in the 4 dogs who completed all 10 drug administrations. Exogenous glucose and insulin were injected at 0 and 20 minutes,

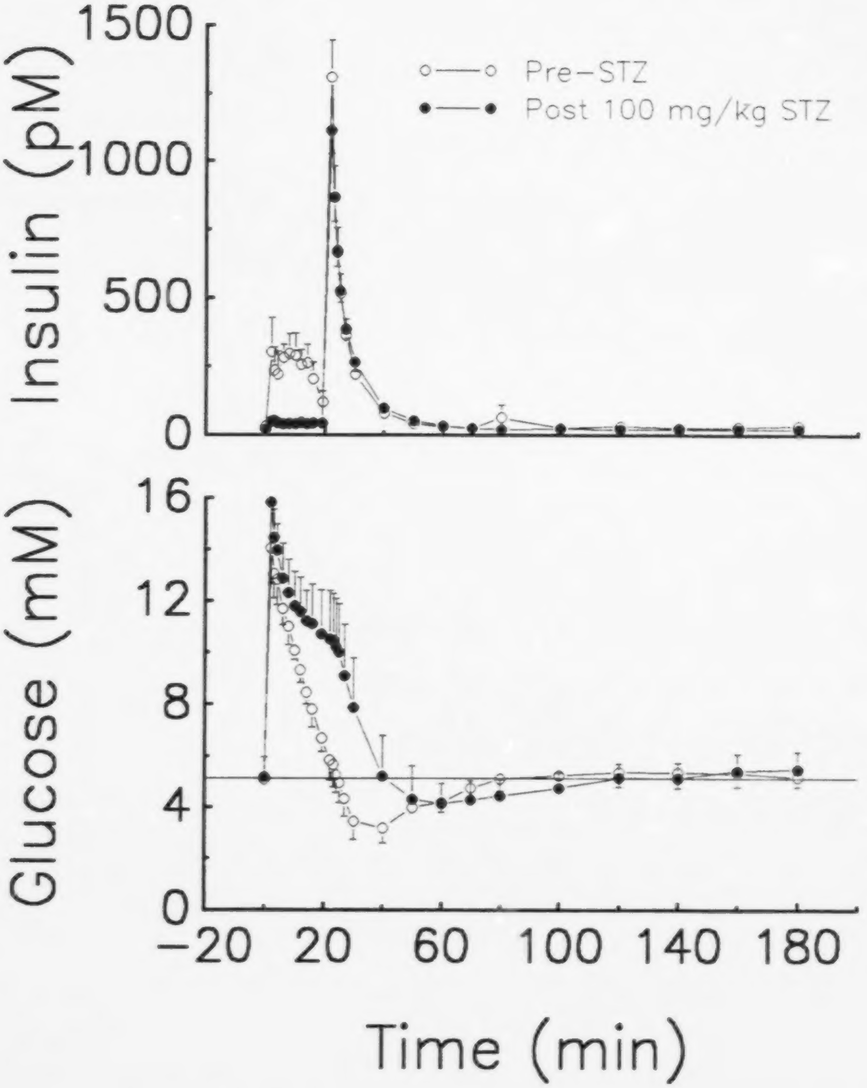


Figure IV-2. Mean glucose and insulin time courses from the FSIGT.

Open circles represent pre-streptozotocin values and closed circles represent values post 100 mg/kg cumulative streptozotocin. Glucose and insulin were injected at 0 and 20 minutes, respectively. Values are means ± STD (n=4).

respectively. The impairment in the insulin secretory response (0-19 minutes) attained by completion of the investigation is clearly illustrated in this figure.

Glucose Tolerance

Glucose tolerance (K_G) consistently deteriorated following each streptozotocin treatment, so that by culmination of the study K_G was only 32% of the pre-streptozotocin value (p=0.003, figure 3). Not surprisingly, this fall in K_G inversely correlated with the dose level (r=-0.72, p=0.0001) and positively correlated with the integrated insulin response to glucose (r=0.54, p=0.0001), but an even stronger correlation was found between K_G and glucose effectiveness (r=0.78, p=0.0001, figure 4).

Results from the stepwise linear regression procedure indicated that glucose effectiveness played the most important role in determining glucose tolerance. When K_G was entered into the model equation as the dependent variable and fasting plasma glucose (FPG), fasting plasma insulin (FPI), the integrated insulin response to glucose (IIRG), insulin sensitivity (S_I), and glucose effectiveness (S_G) were entered as the independent variables the final regression equation was: $K_G = (4.04) + (0.78\ S_G) + (0.0004\ \text{IIRG}) + (-0.46\ \text{FPG}) + (-0.04\ \text{FPI}) + (-0.005\ S_I)$. Table 3 displays the standard errors for each independent variable, the significance of the contribution of each variable in the equation, and the R^2 for each variable (the total variance in K_G which is "explained" by that independent variable in the regression

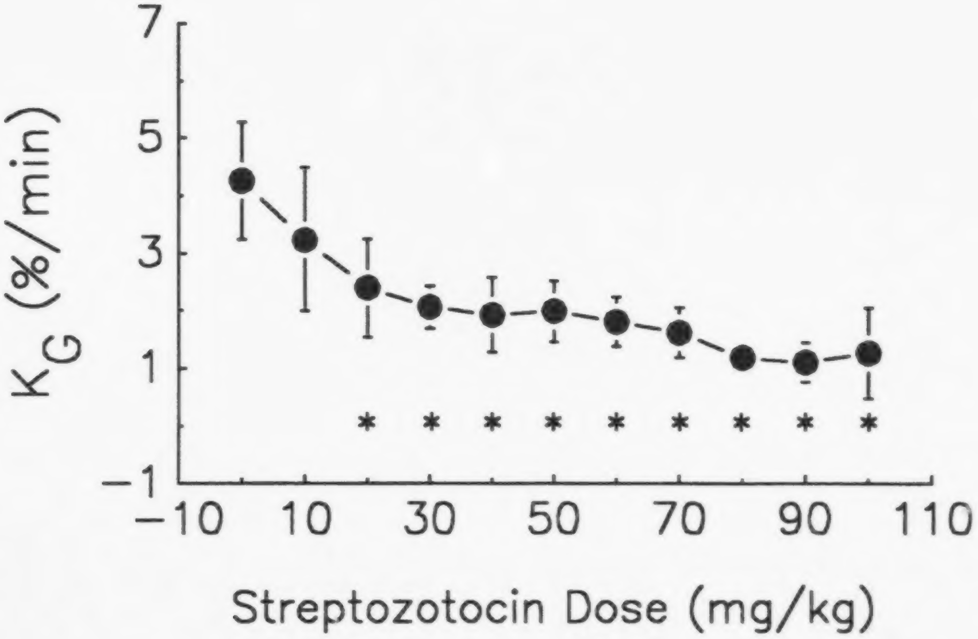


Figure IV-3. Glucose tolerance (K_G) as a function of cumulative streptozotocin dose.

Significant differences from pre-streptozotocin values are indicated via * ($p<0.01$). Values are means ± STD.

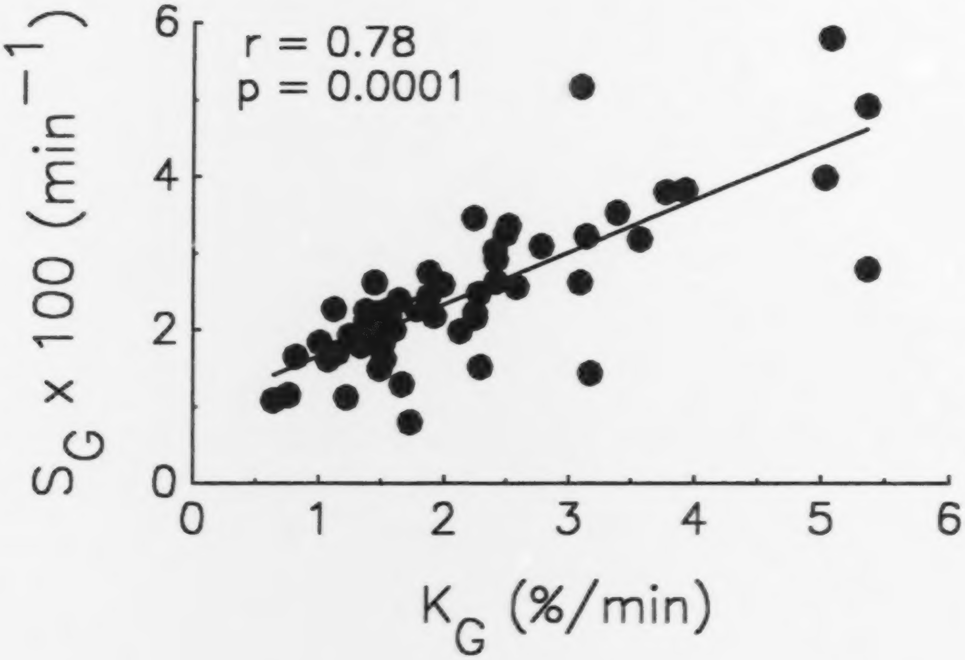


Figure IV-4. Relationship between glucose tolerance (K_G) and glucose effectiveness (S_G).

The regression for all data is illustrated by the solid line (r=0.78, p=0.0001).

Table IV-3. Contribution of parameters of glucose tolerance.

Independent Variable	Equation Value	Standard Error	Significance Level	R^2
Intercept	4.04	0.78	-	-
S_G	0.78	0.11	0.0001	0.46
IIRg	0.0004	0.00006	0.0001	0.16
FPG	- 0.46	0.14	0.0001	0.11
FPI	- 0.04	0.01	0.02	0.03
S_I	- 0.005	0.003	0.06	0.02

The data in this table was derived using the stepwise linear regression procedure, where K_G was entered into the model as the dependent variable. Numbers in the second column represent model equation values for the independent variables tested. The independent variables are listed in the order in which they entered into the model. The significance level for entry into the model, as well as for staying in the model, was $p<0.15$. Abbreviations are defined as follows: S_G, glucose effectiveness; IIRg, integrated insulin response to glucose; FPG, fasting plasma glucose; FPI, fasting plasma insulin; S_I, insulin sensitivity.

equation). The rank order of the significance of the contribution of each independent variable towards the determination of the dependent variable (K_G) is as follows: 1) glucose effectiveness, 2) insulin secretion, 3) fasting plasma glucose, 4) fasting plasma insulin, and 5) insulin sensitivity.

Integrated Insulin Response to Glucose

Figure 5 illustrates the impairment in the integrated insulin response to glucose, as a function of streptozotocin dose. The measure of insulin secretory function at each dose level was significantly different from the pre-treatment value of insulin secretion ($p<0.04$). However, when the insulin secretory response at each streptozotocin dose was contrasted to the response at the successive dose, significance was only observed between the 0 and 1st, 1st and 2nd, and 2nd and 3rd streptozotocin doses ($p<0.04$). In order to determine if the decline in insulin secretory function continued subsequent to the 3rd streptozotocin dose, we contrasted the secretion value at the 20 mg/kg streptozotocin level to all successive dose levels. Significant differences in insulin secretion were found between the 2nd dose and the 3rd through 8th streptozotocin doses ($p<0.04$). This indicates that even though later doses of streptozotocin did not impair beta cell function to the same extent as the initial doses, the impairment did persist throughout the study.

A lack of fit (LOF) test confirmed the non-linearity of the decline in the integrated insulin response to glucose (R^2=57.9%, p=0.000). Figure

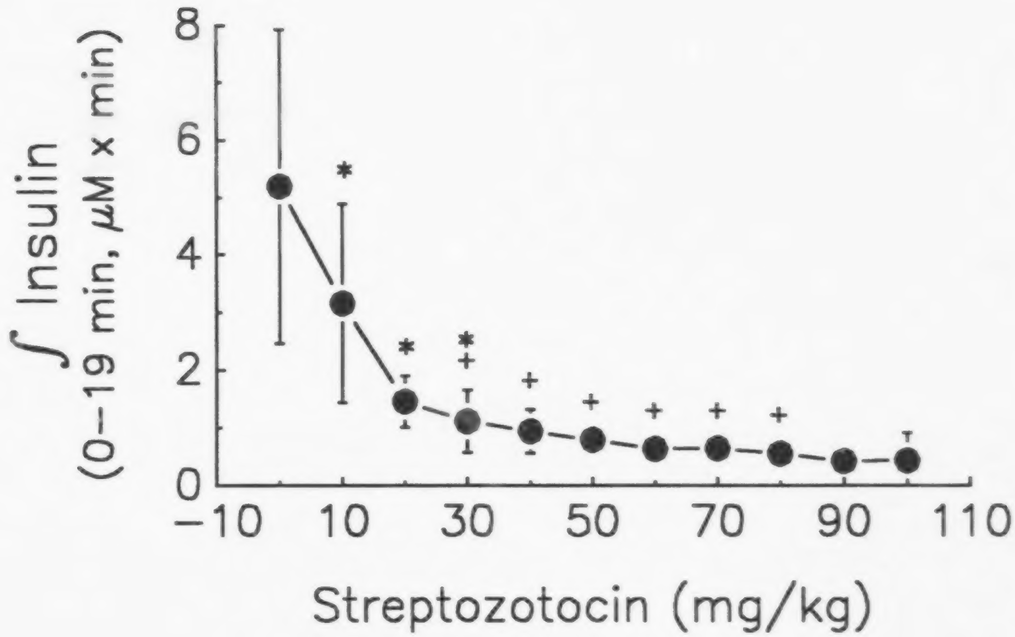


Figure IV-5. Integrated insulin response to glucose as a function of cumulative streptozotocin dose.

The * indicates a significant difference between that streptozotocin dose level and the previous level. The + indicates a significant difference between that streptozotocin level and the 20 mg/kg dose level. Values are means ± STD ($p<0.05$).

6 shows the log of the integrated insulin response to glucose as a function of streptozotocin dose. Visually, it appears that the decrease in secretion may be a log function. However, the LOF test confirmed a curvature to this relationship (LOF R^2=87.5%, p=0.001), indicating that the impairment in insulin secretion is not a log function.

Acute Insulin Response to Glucose

The AIRg is a second measure of insulin secretion which can be obtained from the FSIGT protocol. General trends in the AIRg correlate well with those of the integrated insulin response to glucose, across all streptozotocin dose levels (r=0.99, p=0.0001). The AIRg at each dose level subsequent to the 10 mg/kg streptozotocin level, was significantly different from the pre-treatment value of AIRg (p<0.04). However, when AIRg at each streptozotocin dose was contrasted to the response at the successive dose, significance was achieved only between the 10 and 20 mg/kg streptozotocin dose levels (p=0.03, figure 7). Contrasts of the secretion value at the 20 mg/kg streptozotocin level to all subsequent dose levels revealed significant differences between this level and the 40 through 100 mg/kg dose levels (p<0.05). Results from these statistical comparisons indicate that insulin secretory dysfunction persists throughout the study.

Insulin Sensitivity

As portrayed in figure 8, insulin sensitivity did not change significantly throughout the study period (p>0.21). It is interesting

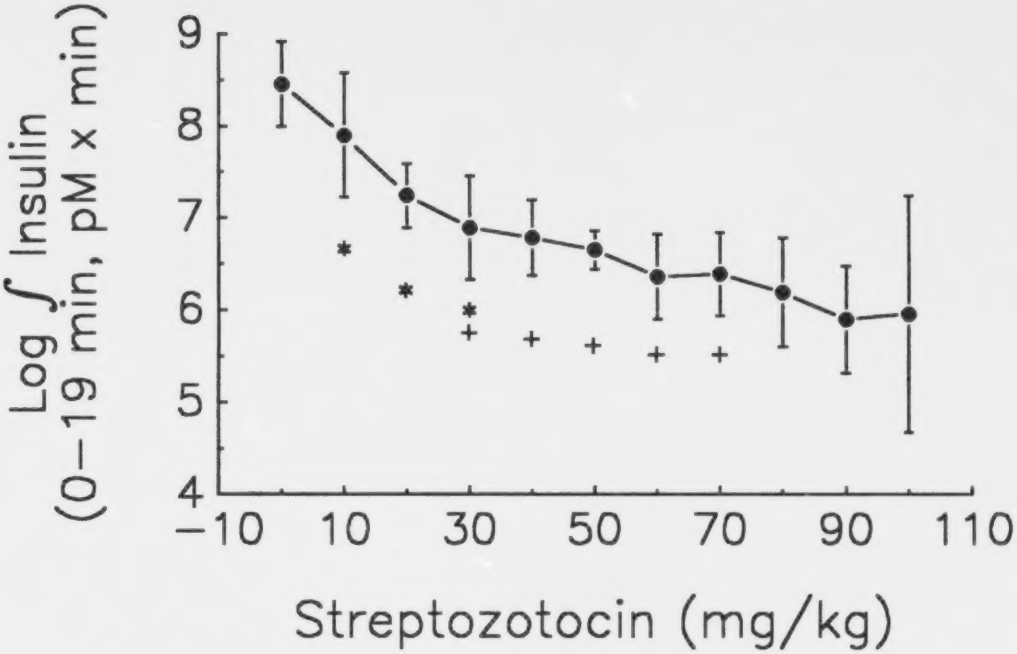


Figure IV-6. Log of the integrated insulin response to glucose as a function of cumulative streptozotocin dose.

The * indicates a significant difference between that streptozotocin dose level and the previous level. The + indicates a significant difference between that streptozotocin level and the 20 mg/kg dose level. Values are means ± STD ($p<0.05$).

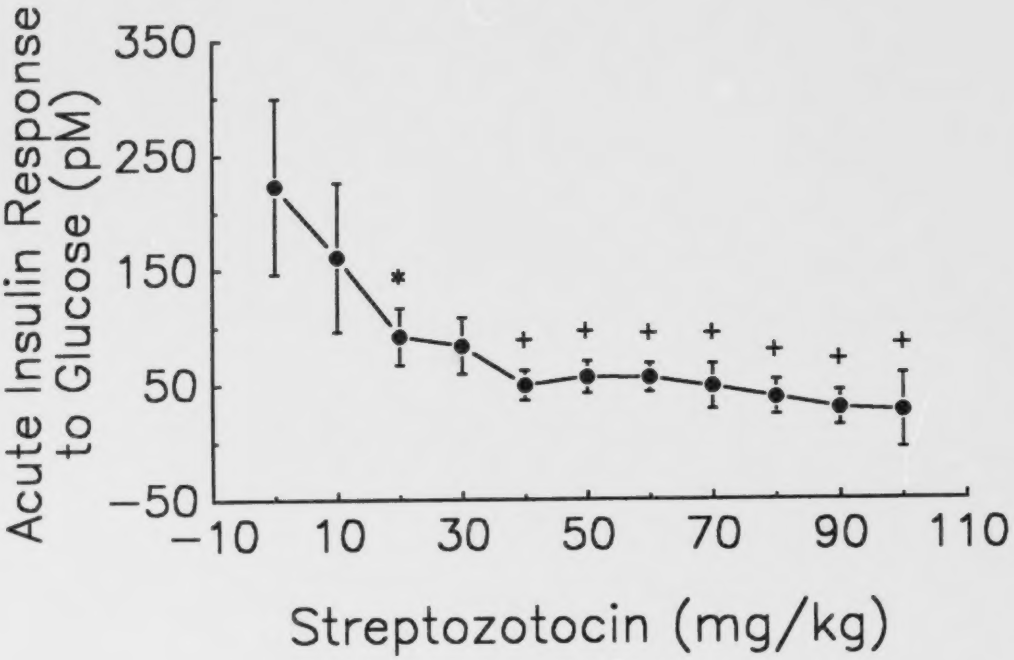


Figure IV-7. Acute insulin response to glucose as a function of cumulative streptozotocin dose.

The * indicates a significant difference between that streptozotocin dose level and the previous level. The + indicates a significant difference between that streptozotocin level and the 20 mg/kg dose level. Values are means ± STD ($p<0.05$).

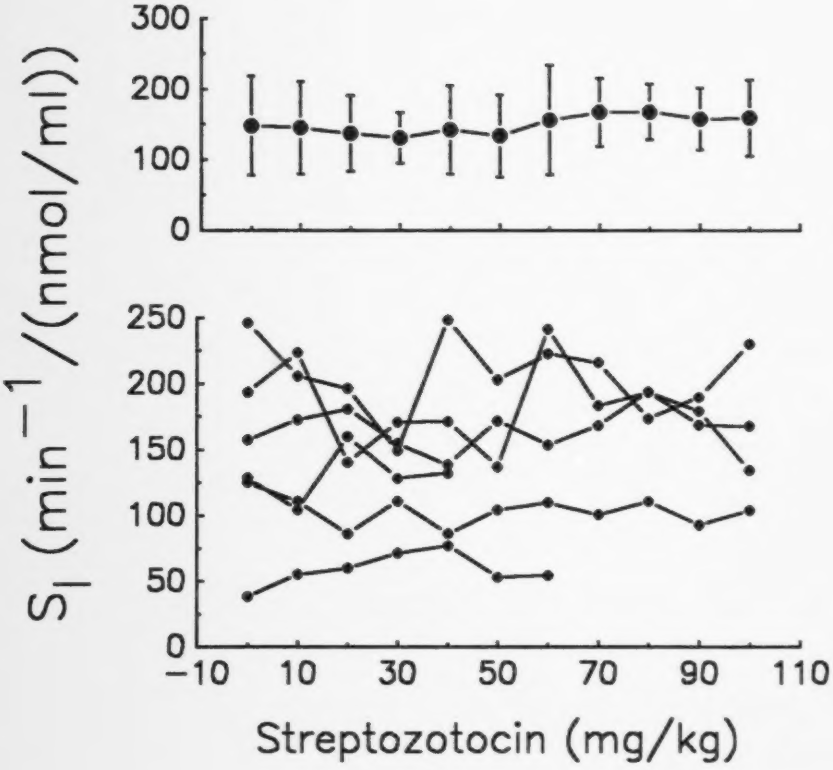


Figure IV-8. Insulin sensitivity (S_I) as a function of cumulative streptozotocin dose.

Average values for S_I are displayed in the top panel and individual animal values are shown in the bottom panel. No significant differences were found. Values in the top panel are means ± STD.

to note that the one animal who became hyperglycemic during the study was also the animal with the lowest S_I.

Correlation analysis revealed a significant inverse relationship between S_I and basal insulin ($r=-0.71$, $p=0.0001$; figure 9). Further analysis revealed, however, that this relationship was mostly due to a difference in mean S_I and basal insulin between dogs as opposed to changes within each dog.

Glucose Effectiveness

In contrast to S_I, glucose effectiveness decreased as the insulin secretory function declined. Significant differences were displayed between S_G at the pre-streptozotocin level and the 1st, 7th, 8th, 9th, and 10th dose levels ($p<0.04$, figure 10). No significant differences were found in the fractional standard deviations for S_G throughout the study ($p=0.0699$).

By completion of the study, S_G had declined to 45% of the pre-treatment value. It should be noted that the net percentage decrease in S_G was slightly greater than 55% when considering only the 4 dogs who completed all 10 streptozotocin doses (table 4). I have chosen to report the $n>4$ percentages, since these values are very similar to percentage changes calculated based on $n=4$ and more importantly, this calculation would seem to be more representative of the general population.

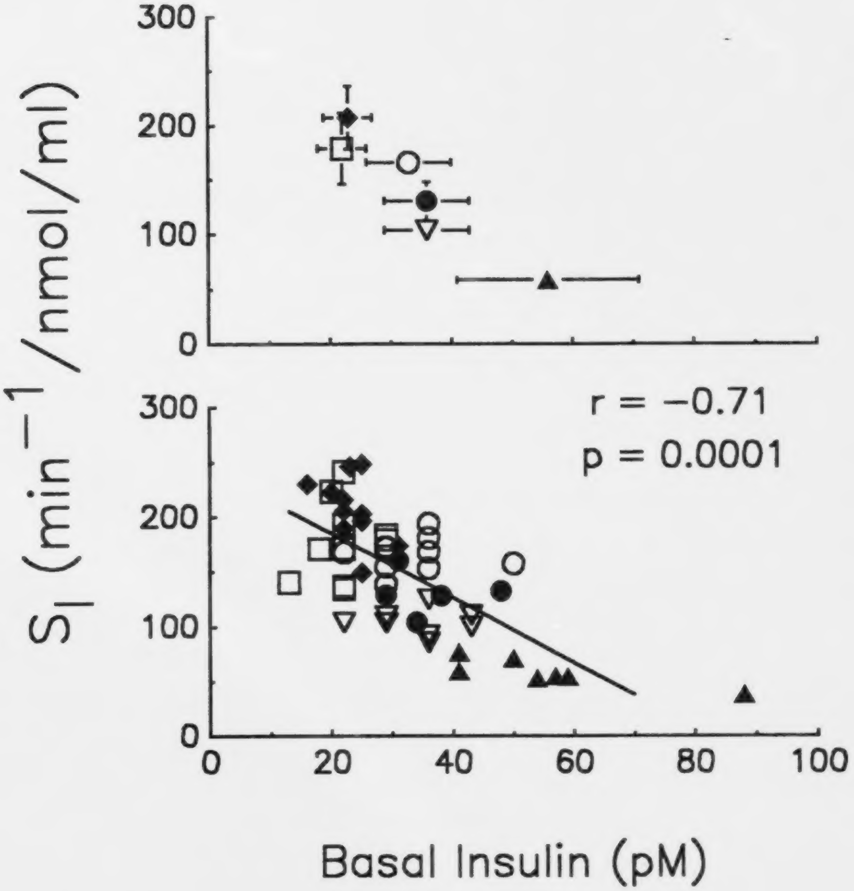


Figure IV-9. Relationship between insulin sensitivity (S_I) and basal plasma insulin.

The top panel displays values as the mean ± STD for each animal. In the bottom panel, like symbols represent data from one animal. The regression for all data is illustrated by the solid line (r=-0.71, p=0.0001). This correlation represents differences between dogs and not differences within individual animals.

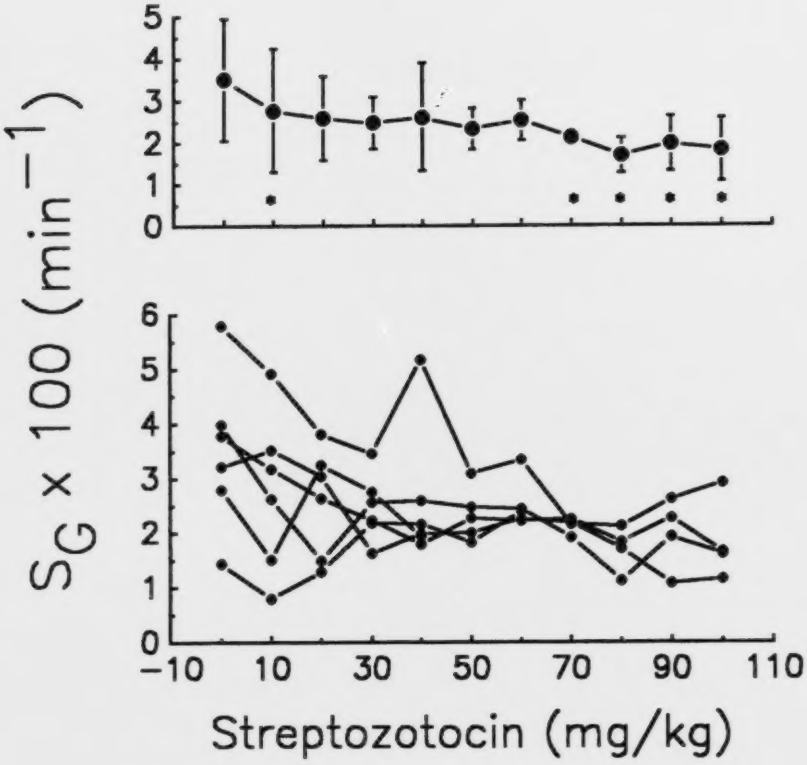


Figure IV-10. Glucose effectiveness (S_G) as a function of cumulative streptozotocin dose.

Average values for S_G are displayed in the top panel and individual animal values are shown in the bottom panel. Significant differences from pre-streptozotocin values are indicated via * ($p<0.04$) in the top panel. Values in the top panel are means ± STD.

Table IV-4. Percent reductions in glucose tolerance, glucose effectiveness, and insulin secretion.

Parameter	Calculated Based on n=4	Calculated Based on n=5	Calculated Based on n=6
K_G	71 ± 11	-	68 ± 13
S_G	58 ± 5	-	55 ± 12
IIRg	86 ± 14	-	87 ± 12
AIRg	85 ± 16	-	86 ± 15
AIRarg at basal glycemia	89 ± 8	87 ± 8	-
AIRarg at hyperglycemia	91 ± 7	90 ± 7	-
Slope	95 ± 7	93 ± 7	-

Numbers represent the percent reduction from the pre-streptozotocin values (n=4, 5, or 6) to the values post 100 mg/kg cumulative streptozotocin (n=4). Values represent means ± STD. Abbreviations are defined as follows: K_G, glucose tolerance; S_G, glucose effectiveness; IIRg, integrated insulin response to glucose; AIRg, acute insulin response to glucose; AIRarg, acute insulin response to arginine; slope, slope of potentiation.

As was indicated previously in the results section pertaining to glucose tolerance, a strong correlation was displayed between K_G and S_G (figure 4). Furthermore, in comparison to the other parameters of glucose tolerance, changes in S_G were discovered to contribute the most significantly to alterations in glucose tolerance.

C. SLOPE OF GLUCOSE POTENTIATION

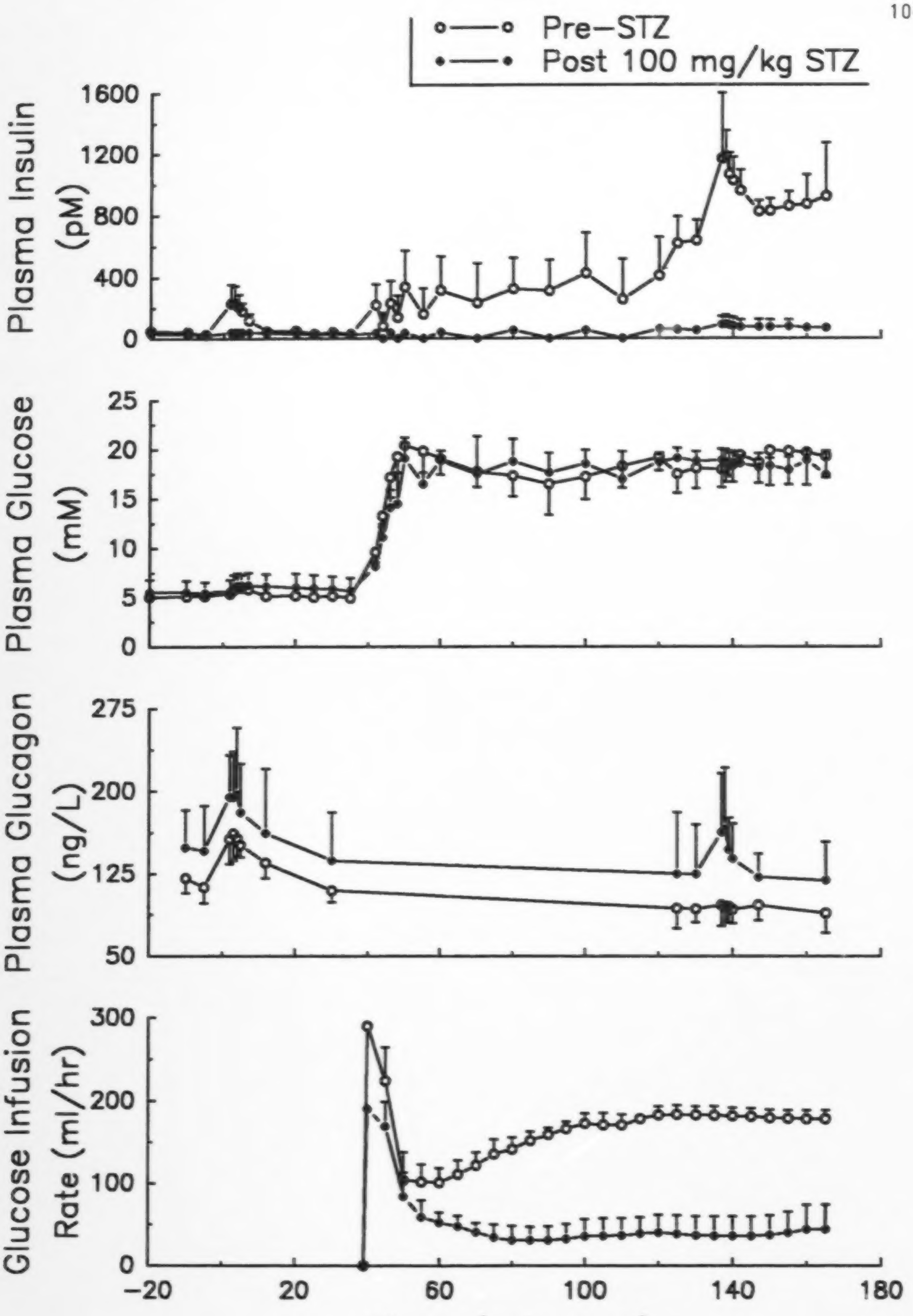
Figure 11 depicts the mean slope of potentiation time courses for plasma glucagon, plasma insulin, plasma glucose and the glucose infusion rate, prior to streptozotocin treatment and subsequent to the final streptozotocin dose, in the 4 dogs who completed all 10 drug administrations. This figure clearly illustrates the impaired insulin secretory response to arginine by the conclusion of the study. As well, the difference in the pre and post glucose infusion rates necessary to induce hyperglycemia are clearly evident.

Acute Insulin Response to Arginine

The AIRarg at basal plasma glucose and at hyperglycemia displayed similar trends as that seen in the AIRg when contrasts were made to pre-streptozotocin insulin secretion values (Figure 12). When AIRargs were contrasted to values at successive dose levels, a significant difference in AIRarg at basal glucose was only observed between the pre-streptozotocin dose and the 10 mg/kg dose ($p=0.008$). Successive contrasts of AIRarg at hyperglycemia revealed significant differences between the 1st and 2nd and the 2nd and 3rd streptozotocin dose levels ($p<0.03$). As was done with the secretion measures from the FSIGT, the

Figure IV-11. Mean insulin, glucose, and glucagon time courses and glucose infusion rates from the slope of potentiation protocol.

Open circles represent pre-streptozotocin values and closed circles represent values post 100 mg/kg cumulative streptozotocin. Arginine was injected at 0 and 135 minutes. A variable rate glucose infusion commenced at 40 minutes to maintain blood glucose at a steady state of approximately 19.6 mM. Values are means ± STD (n=4).



o—o Pre-STZ
•—• Post 100 mg/kg STZ
Plasma Insulin (pM)
1600
1200
800
400
0
Plasma Glucose (mM)
25
20
15
10
5
0
Plasma Glucagon (ng/L)
275
200
125
50
Glucose Infusion Rate (ml/hr)
300
200
100
0
-20
20
60
100
140
180
Time (minutes)

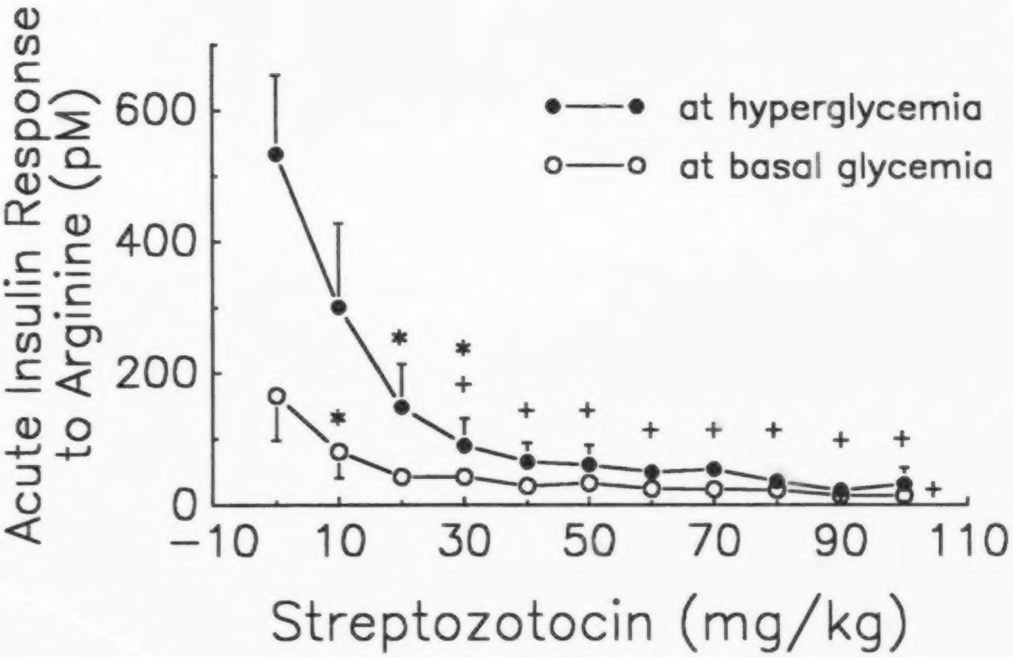


Figure IV-12. Acute insulin responses to arginine at basal glycemia and at hyperglycemia, as a function of cumulative streptozotocin dose.

Open circles represent values at basal glycemia and closed circles represent values at hyperglycemia. The * indicates a significant difference between that streptozotocin dose level and the previous level. The + indicates a significant difference between that streptozotocin level and the 20 mg/kg dose level. Values are means ± STD ($p<0.05$).

values at the 20 mg/kg streptozotocin dose level were contrasted to all subsequent dose levels. At basal glycemia, the AIRarg at the 20 mg/kg dose level was only found to be significantly different from the AIRarg at the 100 mg/kg dose level ($p=0.02$). In contrast, at hyperglycemia the AIRarg at the 20 mg/kg dose level was found to be significantly different from the AIRarg at all subsequent dose levels ($p<0.05$).

Slope of Potentiation

The mean steady state plasma glucose concentrations at basal glycemia and at hyperglycemia during the slope of potentiation protocol were 5.0 mM and 18.43 mM, respectively. Figure 13 illustrates the relationships between the AIRarg's and their corresponding plasma glucose concentrations. Results of contrasts made between the slope of potentiation of the AIRarg values at each streptozotocin dose level and the pre-treatment level were similar to those of the other secretion measures (Figure 14). Successive contrasts demonstrated significant differences exclusively between the 10 and 20 mg/kg streptozotocin dose levels ($p=0.04$). No significant differences were found when the slope value at the 20 mg/kg level was contrasted to subsequent dose levels ($p>0.06$).

Correlation of Insulin Secretory Measures

Table 5 displays the correlation coefficients between all measures of insulin secretory function. Most importantly, the IIRg and the AIRg from the FSIGT were significantly correlated with all three measures of secretion derived from the slope of potentiation protocol (figures 15

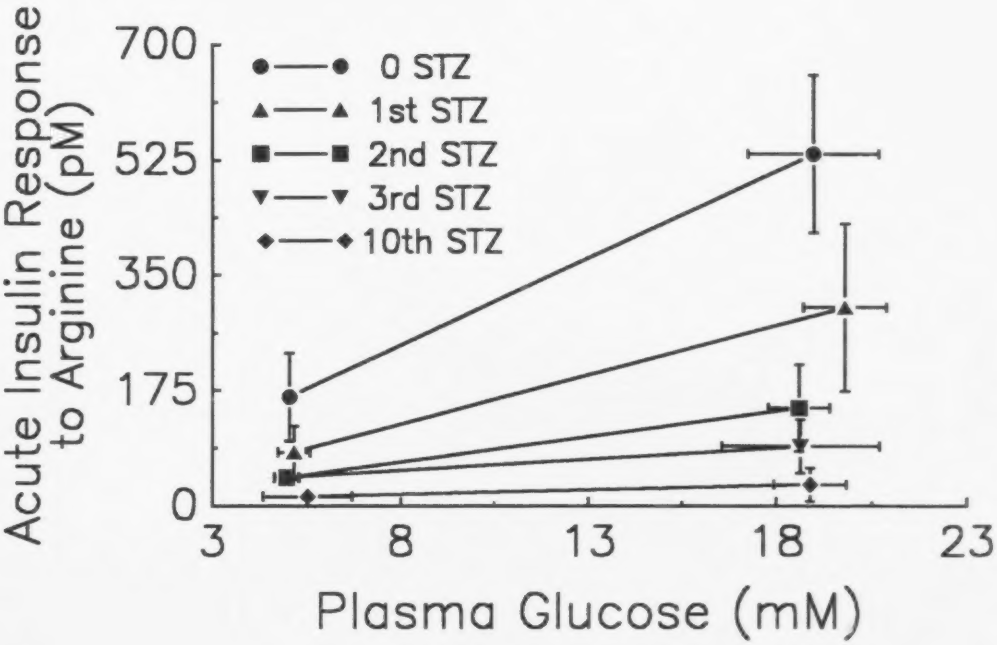


Figure IV-13. Relationships between the acute insulin responses to arginine and their corresponding plasma glucose concentrations.

Only slopes at the 0, 1st, 2nd, 3rd, and 10th streptozotocin dose levels are represented in this figure.

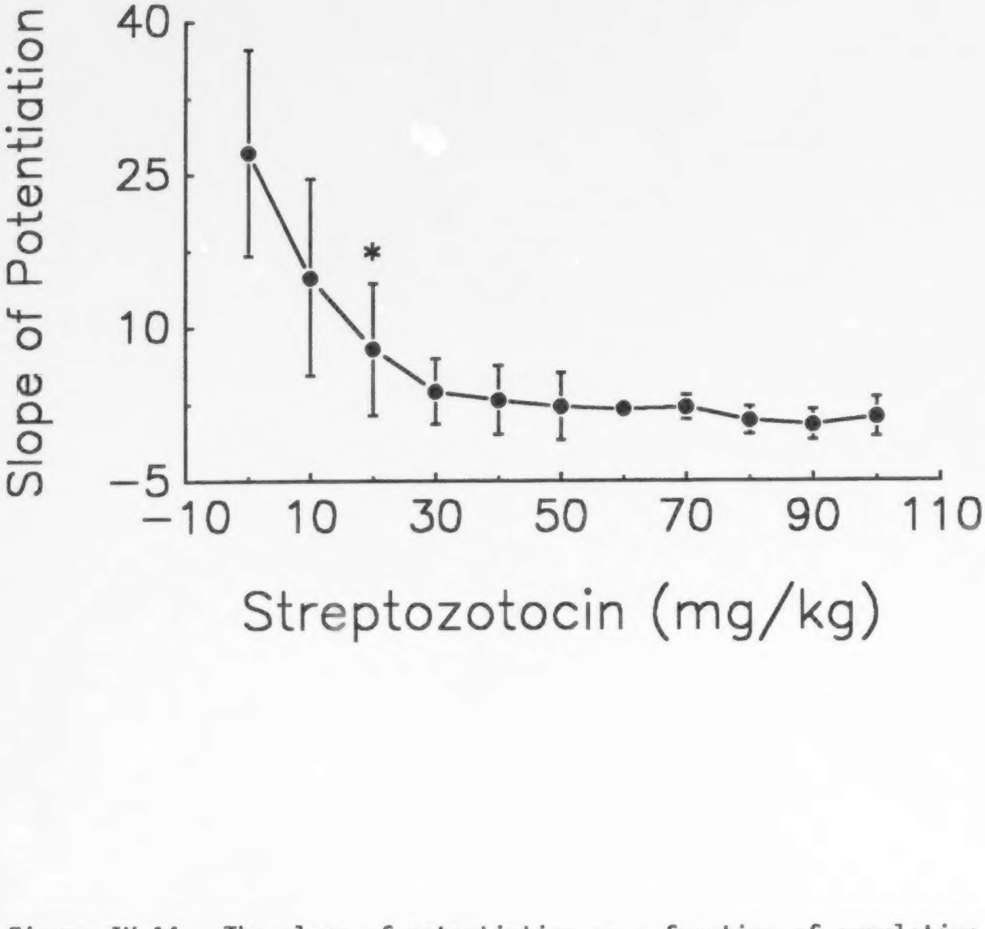


Figure IV-14. The slope of potentiation as a function of cumulative streptozotocin dose.

The * indicates a significant difference between that streptozotocin dose level and the previous level (p=0.04). There were no significant differences between the 20 mg/kg streptozotocin dose level and any of the subsequent dose levels. Values are means ± STD.

Table IV-5. Correlation coefficients of secretion measures.

	AIRg	AIRarg at basal	AIRarg at hyperglycemia	Slope
IIRg	r = 0.99 p = 0.0001 n = 56	r = 0.84 p = 0.0001 n = 49	r = 0.94 p = 0.0001 n = 49	r = 0.86 p = 0.0001 n = 49
AIRg	-	r = 0.87 p = 0.0001 n = 49	r = 0.93 p = 0.0001 n = 49	r = 0.83 p = 0.0001 n = 49
AIRarg at basal glycemia	-	-	r = 0.85 p = 0.0001 n = 49	r = 0.70 p = 0.0001 n = 49
AIRarg at hyperglycemia	-	-	-	r = 0.96 p = 0.0001 n = 49

Abbreviations are defined as follows: IIRg, integrated insulin response to glucose; AIRg, acute insulin response to glucose; AIRarg, acute insulin response to arginine; slope, slope of potentiation.

Figure IV-15. Relationships between the integrated insulin response to glucose (IIRg) and the three measures of insulin secretion derived from the slope of glucose potentiation protocol.

The top panel illustrates the correlation between the IIRg and the acute insulin response to arginine (AIRarg) at basal glycemia (r=0.84, p=0.0001). The middle panel shows the relationship between the IIRg and the AIRarg at hyperglycemia (r=0.94, p=0.0001) and the bottom panel displays the IIRg vs. the slope of potentiation (r=0.86, p=0.0001).

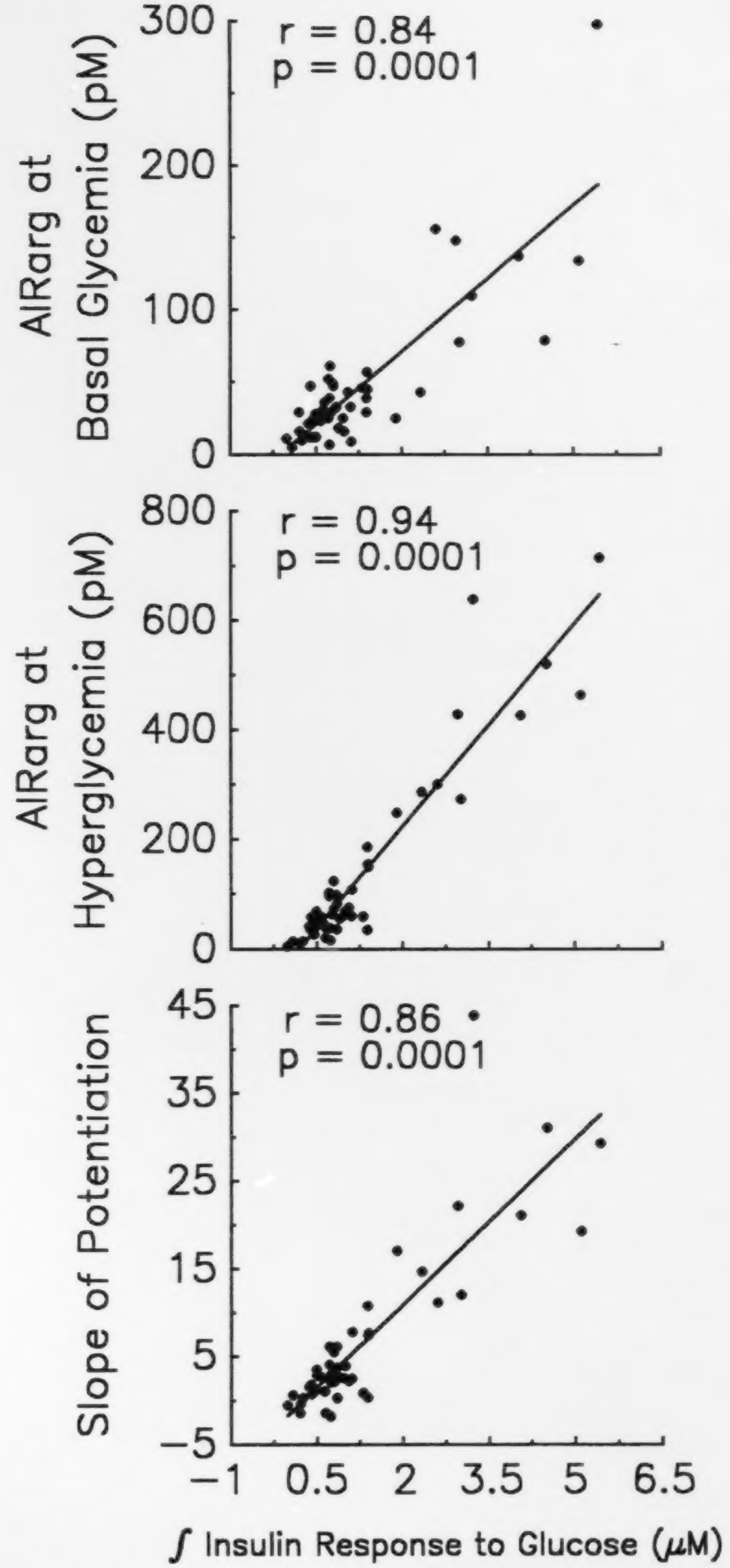
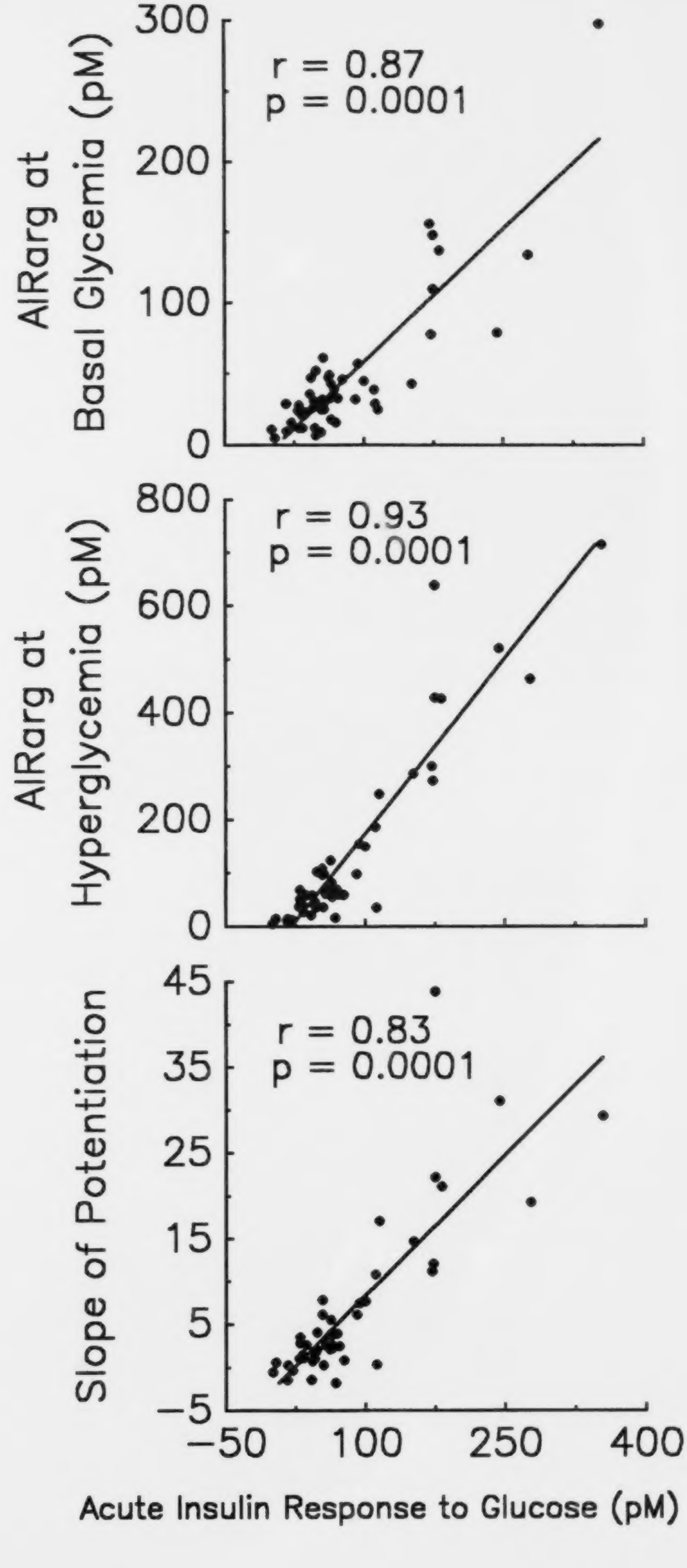

r = 0.84
p = 0.0001
AIRarg at
Basal Glycemia (pM)
300
200
100
0
r = 0.94
p = 0.0001
AIRarg at
Hyperglycemia (pM)
800
600
400
200
0
r = 0.86
p = 0.0001
Slope of Potentiation
45
35
25
15
5
−5
−1
0.5
2
3.5
5
6.5
∫ Insulin Response to Glucose (μM)

Figure IV-16. Relationships between the acute insulin response to glucose (AIRg) and the three measures of insulin secretion derived from the slope of glucose potentiation protocol.

The top panel illustrates the correlation between the AIRg and the acute insulin response to arginine (AIRarg) at basal glycemia (r=0.87, p=0.0001). The middle panel shows the relationship between the AIRg and the AIRarg at hyperglycemia (r=0.93, p=0.0001) and the bottom panel displays the AIRg vs. the slope of potentiation (r=0.83, p=0.0001).


300
200
100
0
AIRarg at
Basal Glycemia (pM)
r = 0.87
p = 0.0001
800
600
400
200
0
AIRarg at
Hyperglycemia (pM)
r = 0.93
p = 0.0001
45
35
25
15
5
−5
Slope of Potentiation
r = 0.83
p = 0.0001
−50
100
250
400
Acute Insulin Response to Glucose (pM)

and 16).

Table 4 shows the total percent decrease in insulin secretion by the end of the study for each measure of beta cell function. The overall decline in the insulin secretory response was between 86 and 93%.

Integrated Glucagon Response to Arginine

Figure 17 displays the mean integrated glucagon responses to arginine at both basal glycemia and hyperglycemia during the slope of potentiation protocol. Contrasts between values at successive dose levels revealed significant differences only at basal glycemia, between the 0 and 1st and the 9th and 10th streptozotocin doses ($p<0.02$). Univariate testing for within subject effects, however, showed no significant changes in the glucagon responses at either basal glycemia or hyperglycemia ($p=0.31$ and $p=0.30$, respectively).

D. SUMMARY

Of the six dogs studied, 4 received 10 streptozotocin doses without developing fasting hyperglycemia, 1 animal developed fasting hyperglycemia following 6 doses, and 1 animal died of severe glomerulonephritis following the 5th streptozotocin dose. The glucose tolerance of the dogs gradually declined throughout the study period, reaching a 68% reduction by completion of the study. Following the first two doses of streptozotocin, dramatic reductions in insulin secretion were observed. Though later doses of the drug did not impair beta cell function to the same extent as the initial doses, the

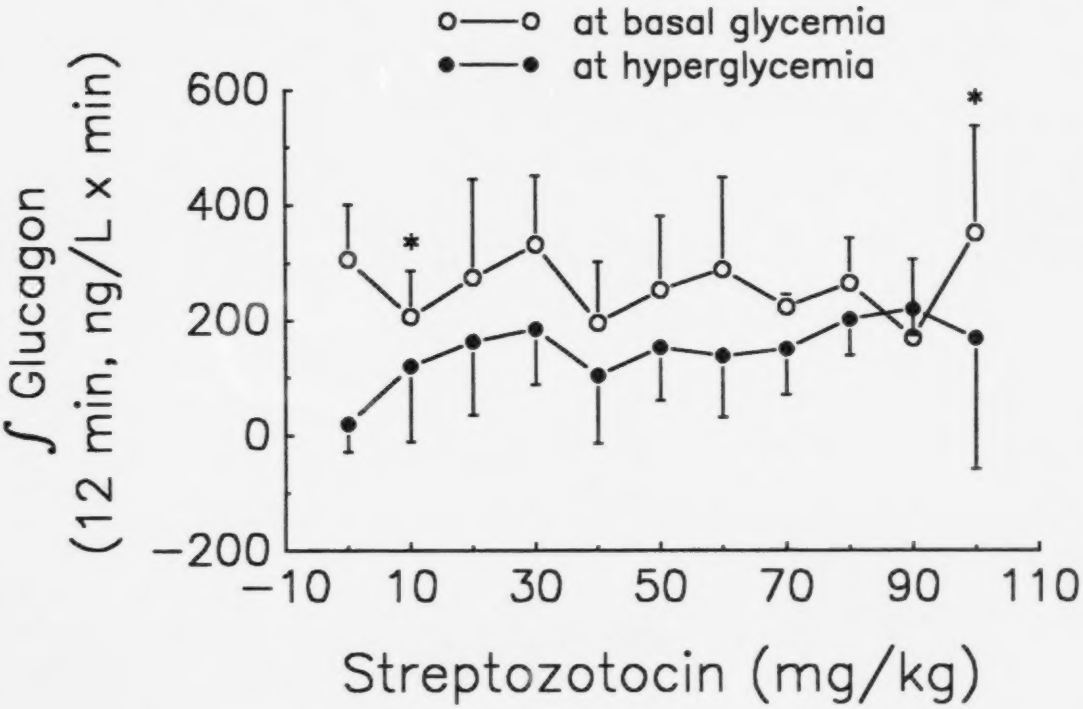


Figure IV-17. Integrated glucagon response to arginine at basal glycemia and at hyperglycemia, as a function of cumulative streptozotocin dose.

The open circles represent values at basal glycemia and the closed circles represent values at hyperglycemia. The * indicates a significant difference between that streptozotocin level and the previous level ($p<0.02$). Values are means ± STD.

impairment in insulin secretory capacity did persist. By completion of the study, the decrease in the insulin response was between 86 and 93%, as indicated by all measures of insulin secretion. Despite this severe reduction in the insulin secretory response, fasting plasma insulin, fasting plasma glucose, and insulin sensitivity did not change significantly. In contrast, glucose effectiveness was reduced to 45% of pre-streptozotocin values by the end of the study. Furthermore, it was determined that the contribution of glucose effectiveness to the total variance in glucose tolerance was 188% greater than the contribution of insulin secretion.

In addition, these studies demonstrate that the integrated and acute insulin responses to glucose from the FSIGT are significantly correlated to the AIRarg at basal glycemia, the AIRarg at hyperglycemia, and the slope of potentiation of the AIRarg. These correlations were maintained over a range of approximately 7 - 100% beta cell function.

V. DISCUSSION

A. MODEL OF SUBCLINICAL BETA CELL DYSFUNCTION

Background

Six theoretical stages of IDDM have previously been described (46). During the fourth stage, which immediately precedes the clinical onset of IDDM, insulin secretory function progressively declines (46), while glucose tolerance generally remains normal (47). The progressive deterioration of beta cell function can take place over several years prior to the manifestation of clinical symptoms (60, 125). Since little research has been performed to investigate the "pre-diabetic" period, the relationship between subclinical insulin deficiency and the factors determining glucose tolerance are not well described.

Though animal models of spontaneous diabetes do exist, these models are not suitable for investigating pre-clinical IDDM. Due to the rapid onset of the disease in these animals (88, 91), time is not available to study the longitudinal effects of progressive beta cell dysfunction. Other available animal models of diabetes, such as those which induce diabetes via partial or total pancreatectomy or via diabetogenic doses of a beta cell toxin, do not result in progressive beta cell loss. Needless to say, animal models of diabetes have made a limited contribution toward our understanding of the effects of a progressive impairment in insulin secretory function on the determinants of normal glucose homeostasis. An animal model of progressive beta cell dysfunction would be a valuable tool to investigate the mechanisms underlying the metabolic alterations associated with a gradual deterioration of beta cell function. Hence, the first specific aim of this thesis project was to establish an animal model of progressive insulin secretory dysfunction.

Repeated Low Dose Streptozotocin Model

Repeated low doses of 10 mg/kg streptozotocin were administered to canines in attempts to induce a gradual decline in beta cell function. By treating the animals with the beta cell toxin on a bi-weekly basis, it was possible to evaluate the insulin secretory function subsequent to each drug dose. Thus, in this model, there was adequate time available to conduct a longitudinal investigation of the effects of subclinical insulin deficiency. Results indicated that the decline in beta cell function was not linear, but that the deterioration was most dramatic following the first two streptozotocin doses (figure IV-5). Though later doses of the drug did not impair beta cell function to the same extent as the initial doses, the impairment did persist, as indicated via contrasts between insulin secretion values at the 20 mg/kg streptozotocin dose level and subsequent dose levels.

Since impairment of beta cell function did not progress throughout the entire study, it is recognized that there are limitations to the interpretations which can be made from this investigation due to the dissimilarity between the model and human IDDM. As is the case with other animal models of diabetes, any conclusions derived from the repeated low dose streptozotocin model will reflect the specific conditions associated with the model (e.g. the pattern of decrease in beta cell loss, possible direct effects of streptozotocin independent of it's effects upon the beta cell, etc.). In fact, a portion of the discord between the results from the present investigation and those of other studies, may be related to the dissimilarities between this model of subclinical insulin deficiency and other models of diabetes. As well, the heterogeneity of human diabetes per se, adds a factor of difficulty to interpreting results from animal models of the disease, which in some aspect invariably fall short of truly representing the actual disease. It is also recognized, however, that besides human subject trials, animal models representing human disease states, are the most viable means by which we can make progress in understanding human disease. Though the repeated low dose streptozotocin model of subclinical insulin deficiency may not truly epitomize human IDDM, it may provide some insight into the relationships between the parameters of glucose tolerance, which could possibly be extrapolated to the human condition.

Streptozotocin Resistance

It is not understood why the toxic effect of streptozotocin to induce beta cell destruction, diminishes after the initial drug administrations in this multiple dose regimen. There are four possible explanations for this apparent drug resistance. First, based on the theory that the glucose moiety of streptozotocin facilitates it's selective uptake into the beta cell (44, 53, 66), uptake of the toxin may be decreased. Initial doses of the drug might induce a defect in the glucose monitoring system or in the glucose transport mechanism, thereby, resulting in a decreased uptake of streptozotocin (83). Second, an efficient repair mechanism of streptozotocin induced DNA lesions may exist (83). Third, perhaps not all beta cell populations (24) exhibit identical sensitivities to streptozotocin, such that more "sensitive" populations are destroyed by initial drug administrations. The more "toxin resistant" cell populations would then remain, sustaining normal fasting glycemia. In the present study, the surviving cells within the pancreatic islets of Langerhans were small in size, possibly representing such a "toxin resistant" population. Lastly, there may be some mechanism by which streptozotocin specifically attacks some part of the pathway leading to the acute insulin response to a secretogogue. In contrast, the pathway by which basal insulin release occurs may be resistant to the drug's toxicity, thereby maintaining normal fasting glycemia and the residual insulin secretion in response to a secretogogue. Further investigation, however, will be necessary to determine the actual mechanism responsible for the apparent decline in streptozotocin's action in this multiple dose regimen. At present, the most conclusive statement that can be made is that multiple low doses of streptozotocin are less toxic than a single high dose of the drug, equal to the cumulative total of the low doses (86, 89).

Systemic Toxicity

While it is true that bi-weekly administration of 10 mg/kg streptozotocin did not induce a linear loss of beta cell function, the progression of insulin secretory dysfunction was similar in all dogs; the first two streptozotocin doses produced the most dramatic effects, while latter doses induced lesser, but persistent effects. It appears then, that the repeated low dose streptozotocin model is a relatively reproducible model of subclinical insulin deficiency. In contrast, when high doses of streptozotocin are employed to promote a certain degree of hyperglycemia, to damage a specific percentage of beta cells, or to induce overt diabetes, the outcome appears to be unpredictable. Conventionally, streptozotocin has been used in single high doses or multiple low doses administered for 3-5 consecutive days. The single intravenous dose of streptozotocin required to induce overt diabetes approximates the LD_{50} dose level, which is estimated to lie between 25 and 50 mg/kg in dogs (113, 116). Since the diabetogenic dose and the LD_{50} dose vary between and within species (115, 116), the outcome of administering a high dose of streptozotocin is not only unpredictable, but even includes the risk of death.

In comparison to the toxic effects of a single diabetogenic dose of streptozotocin, the multiple low dose regimen used in the present investigation appears to be associated with a lower risk of systemic toxicity. While 4 of the dogs in the present study received 10 streptozotocin doses (10 mg/kg streptozotocin per dose) without becoming hyperglycemic, one animal became overtly diabetic following the 6th drug treatment and one animal died following the 5th dose.

Animal Complications

The dog who became overtly diabetic exhibited a lower pre-streptozotocin treatment value of insulin sensitivity when compared to the pre-study insulin sensitivity values displayed in the other dogs (38.34 min^{-1}/(nmol/ml) vs. the mean of the other dogs, 169.93 min^{-1}/(nmol/ml)). Since this dog appeared to weigh more than it's probable ideal body weight, it is speculated that the observed insulin resistance was associated with the obesity (110). As beta cell function deteriorated, the insulin sensitivity of this animal was probably insufficient to support the decrease in insulin secretion; i.e. the product of insulin sensitivity and insulin secretion fell below the threshold essential to maintain normal fasting blood glucose. In contrast, the animals which did not develop hyperglycemia, were probably maintained in a normoglycemic state as a result of the combined effects of insulin action, glucose effectiveness, and the remaining insulin secretion.

In the dog who died following the 5th streptozotocin dose, the drug itself may have been the causative agent in the development of the fatal bout of acute glomerulonephritis. Though the specific ages of the dogs were not known, this particular one appeared to be older than the other dogs studied and therefore, may have been more susceptible to the toxic effects of the streptozotocin. Since the blood urea nitrogen (BUN) and creatinine levels were within the normal limits even up until immediately prior to this animal's last streptozotocin administration, the acute damage most likely occurred within the two days between the last streptozotocin dose and death. Though necropsy results of the other dogs also revealed a degree of kidney damage, they too failed to display abnormalities in BUN or creatinine.

Summary

The repeated low dose streptozotocin model provides a novel approach to study the longitudinal effects of subclinical insulin deficiency in canines. Since the pathogenesis of human IDDM is believed to commence with the genetic susceptibility of an individual, followed by a "triggering" event, which results in active autoimmune mechanisms (46) precipitating linear beta cell destruction (70, 125, 157), it is recognized that the repeated low dose streptozotocin model is not a true representation of human pre-clinical IDDM. Additionally, the possibility of undefined direct effects of streptozotocin may contribute to dissimilarities between this model of pre-diabetes and physiological IDDM. Nevertheless, it does seem to be the most appropriate animal model currently available to investigate the effects of subclinical insulin deficiency. Data obtained via this model of progressive beta cell dysfunction may provide insight into human pre-clinical IDDM which could ultimately lead to improvements in diabetes intervention programs.

B. EFFECTS OF SUBCLINICAL BETA CELL DYSFUNCTION

The second specific aim of this thesis was to determine the longitudinal relationship between a gradual decline in insulin secretory function and insulin sensitivity, glucose effectiveness, and glucose tolerance.

Previous attempts have been made to define the effects of diminished insulin secretory function on insulin action and glucose effectiveness, yet little is known about what changes, if any, are occurring in these parameters during the pre-clinical phase of human IDDM. Investigations in non-diabetic siblings of IDDM patients have demonstrated that insulin secretion and insulin sensitivity may be reduced in these individuals (71, 96, 112). Studies performed in humans nevertheless, have not been able to distinguish whether this impairment in insulin sensitivity is an inherited or acquired defect (e.g. an effect of impaired insulin secretion). The effect of beta cell dysfunction on glucose effectiveness is not well defined, though some data indicates that a reduction in glucose effectiveness is associated with subclinical insulin deficiency (50, 52, 135).

Insulin Sensitivity

Results from the present investigation indicate that a severe impairment in insulin secretory function can exist without affecting insulin sensitivity. By using the repeated low dose streptozotocin model in canines, it was demonstrated that despite an 86 - 93% reduction in the insulin secretory response to glucose, fasting plasma glucose concentrations and insulin sensitivity did not change. These results, as well as those from other investigations (52, 78, 102, 118, 135), support the hypothesis that impaired beta cell function does not necessarily result in insulin resistance, particularly when glycemia remains within the normal limits. A study by McCulloch and colleagues, however, opposes this theory (98).

As described in chapter I of this thesis, McCulloch and colleagues studied normoglycemic, low dose streptozotocin treated baboons (98). Their results suggest that insulin resistance is directly induced by an impairment in insulin secretion. At one week subsequent to the streptozotocin treatment, animals displayed a 62% decrease in the acute insulin response to glucose and a 54% reduction in insulin sensitivity, while normal fasting glycemia was maintained.

Though the reason for the conflicting data is not clear, streptozotocin treatment regimens and/or experimental protocols may have contributed to the discord. Streptozotocin treatment regimens differed in that the baboons were treated with the drug for 3 consecutive days and were subsequently studied only one week following the initial drug treatment. In contrast, the animals in this thesis project were provided with an 8 day recovery period subsequent to the administration of a much lower dose of streptozotocin. Perhaps, in the baboons, the acute side effects of the toxin were still present during the data collection period. Consequently, their results may reflect a pathological state associated with direct drug induced toxicity.

Another possible and perhaps more critical difference between the present study and that of McCulloch et al. (98), was that in their study the glucose only IVGTT was used instead of the insulin modified FSIGT. Original studies in normal dogs demonstrated that insulin sensitivity values obtained via the glucose only IVGTT correlate well with values derived using the glucose clamp (51). In non-diabetic man, however, the values from the glucose only protocol were poorly correlated with the clamp. Later, it was determined that a bolus of tolbutamide or insulin can be administered during an FSIGT to produce adequate circulating insulin concentrations for improved estimation of insulin sensitivity (9, 36, 50, 153). Since the baboons used in McCulloch et al.'s study would probably be more analogous to the human than the dog with regards to their metabolic response during an IVGTT, and since the glucose only IVGTT was found to be inappropriate for determining insulin sensitivity in humans, the glucose only protocol was probably also inappropriate for use in the baboons. By using that protocol, a portion of the impairment in the insulin secretory function may have been erroneously obscured, while the decrease insulin sensitivity may actually have been a reflection of a defect in glucose effectiveness. Overall, the insulin modified FSIGT would have been a better choice of protocols for accurately estimating insulin secretion and insulin sensitivity.

Effect of Streptozotocin

A last issue to consider with respect to the reason for the conflict between the results of the present study and the study of McCulloch et al. (98) is a direct effect of streptozotocin to induce insulin resistance. Numerous studies have used streptozotocin to induce beta cell destruction and have assumed that the drug does not affect the sensitivity of the insulin target cells to insulin. Several pieces of evidence, however, hint at the possibility that streptozotocin itself does induce insulin resistance. First, changes in hepatocytes, renal tubules, and lymphoid tissue have been demonstrated in animals injected with streptozotocin (5, 86). Second, the structure of streptozotocin seems to be compatible with such a theory. The streptozotocin molecule is a nitrosourea side chain linked to the C2 position of D-glucose (12). Some data supports the idea that the glucose moiety of the streptozotocin molecule is responsible for directing the toxin specifically to the beta cell (44, 53, 83, 84). If this is true, it may be that the glucose component of the streptozotocin molecule also directs it to the glucose transporters in the peripheral tissues, promoting damage at these sites as well.

A third piece of evidence which contributes to the hypothesis that streptozotocin induces insulin resistance, is based on the fact that animals treated with high doses of streptozotocin have displayed insulin resistance prior to the onset of fasting hyperglycemia (25, 98, 136). When diabetes is induced by autoimmune destruction or by partial pancreatectomy, decreased insulin secretion per se does not induce insulin resistance (50, 52, 135). In contrast to the effects of high doses of streptozotocin, the low doses administered in the present study did not cause any alterations in insulin sensitivity. One explanation for this phenomenon may be that larger doses of the drug produce varied or more toxic effects than do lower doses. This idea is conceivable, since it has been shown that single high dose and multiple low dose regimens of streptozotocin treatment involve different mechanisms for beta cell destruction (89, 103). The question remains then, do high doses of streptozotocin induce insulin resistance independent of the drug's effect on the beta cell? Further investigation addressing this question may be warranted since, if streptozotocin is discovered to directly affect insulin sensitivity, it may be necessary to re-interpret many of the studies in which the drug has been used.

Summary

Based on results from the present study, it can be concluded that diminished insulin secretory function and repeated low doses of streptozotocin do not directly affect insulin sensitivity. It is speculated that insulin resistance did not develop for two reasons. First, low doses of streptozotocin do not have a direct toxic effect on the peripheral tissues to induce insulin resistance. Second, hyperglycemia may be necessary to precipitate a decline in insulin sensitivity. This second hypothesis is founded on data from other laboratories which have shown that plasma glucose concentration is closely associated with the regulation of insulin sensitivity (22, 102, 118). With respect to the relationship between insulin secretion and sensitivity, the primary conclusion that can be drawn from this thesis investigation is that normal insulin sensitivity can be maintained in the face of severely reduced beta cell function.

Reduced Glucose Effectiveness

In the present study, despite the maintenance of normal fasting plasma insulin, fasting plasma glucose, and insulin sensitivity, glucose effectiveness declined to 45% of pre-streptozotocin values. Three possible causes for the decline in glucose effectiveness include: 1) an effect of chronic insulin deficiency secondary to beta cell dysfunction, 2) an artifact of the MINMOD calculation of glucose effectiveness (S_G), and 3) a cumulative toxic effect of streptozotocin.

Effect of Chronic Insulin Deficiency

Results from the present study support the idea that a reduction in glucose effectiveness may be an effect of chronic insulin deficiency. One might ask then, at what site and by what mechanism might this "effect" occur? By definition, there are two plausible mechanistic sites for a defect in glucose effectiveness: glucose disposal at the peripheral tissues or suppression of glucose production in the liver. Data from the present investigation does not allow an impairment from one site or the other to be distinguished, but instead provides a composite value of the two components of glucose effectiveness. In the future, experiments using glucose clamps and tracer methodology could be used to identify the contribution of the individual components of glucose action. If such studies were performed in conjunction with the repeated low dose streptozotocin model, the site of the defect in glucose's ability to promote it's own disposal could be disclosed.

Effect of a Calculation Artifact

A second cause for the defect in glucose effectiveness may be related to an artifact inherent in the FSIGT/MINMOD calculation of glucose action, S_G. Two possible areas where artifacts could have been introduced will be presented and also the evidence to dispute their existence.

First, since the effects of basal insulin are incorporated into the MINMOD calculation of S_G, a change in basal insulin could have potentially contributed to an error in S_G determination. There were, however, no significant changes in basal plasma insulin concentrations throughout the study. Therefore, alterations in this parameter could not have been associated with an artifact in the determination of glucose effectiveness.

Second, the calculation of S_G could erroneously incorporate a proportion of insulin mediated glucose uptake, which if reduced, would also result in a decreased S_G. In the present study a progressive decline was observed in the plasma insulin concentrations in response to the glucose injection during the FSIGT. A recent study, however, has demonstrated that suppression of this insulin response does not alter the value of the MINMOD calculation of S_G (49). When somatostatin (an inhibitor of insulin secretion) was used to suppress the insulin response to glucose in the insulin modified FSIGT, it was determined that values of S_G were not significantly different from values where the insulin response was not suppressed (49). Thus, the plasma insulin concentration above basal following the glucose injection in the FSIGT has no apparent effect on the MINMOD determination of glucose effectiveness.

A last point in refute of a possible calculation artifact is that the fractional standard deviations for glucose effectiveness, which indicate how well the parameter is defined by MINMOD, did not change significantly throughout the study.

Effect of Streptozotocin

The last potential reason for the decline in glucose effectiveness is a cumulative toxic effect of streptozotocin. Previous studies performed in our laboratory suggest that the defect is not associated with the streptozotocin treatment (50, 52, 135). In dogs, when beta cell insufficiency was rendered via pancreatectomy/islet autotransplantation (no streptozotocin treatment), results were compatible with findings from this thesis investigation; impaired glucose effectiveness was associated with a severe reduction in insulin secretion (9-29% of control animals), despite normal fasting glucose, fasting insulin, and insulin sensitivity (52, 135). Additionally, in newly diagnosed human IDDM subjects, individuals who entered full clinical remission from IDDM had significantly improved insulin sensitivity values, yet glucose effectiveness and insulin secretion remained chronically impaired (50). In both of these studies, a defect in glucose effectiveness was associated with beta cell loss, in the absence of hyperglycemia and without streptozotocin treatment. Based on these results it is believed that the defect in glucose effectiveness in the present study was not induced by streptozotocin toxicity.

Summary

Results from the present investigation indicate that though normal insulin sensitivity and fasting plasma glucose may be maintained in the presence of a severe deficit in insulin secretion, a significant impairment in glucose effectiveness eventually develops. Though the initial fall in glucose tolerance was predominantly related to the marked loss of beta cell function following the first two streptozotocin treatments, the primary cause of subsequent decreases in glucose tolerance was associated with the defect in glucose action. In fact, the contribution of glucose effectiveness to the total variance in glucose tolerance was 188% greater than the contribution of insulin secretion. Currently, it can only be speculated that the defect in glucose effectiveness is an effect of chronic insulin deficiency. Future investigation to determine the mechanisms responsible for this defect may be valuable in formulating therapies for diabetes intervention.

C. MEASURES OF INSULIN SECRETION

Background

In the past, a variety of secretagogues have been used to promote an acute insulin response for characterization of beta cell function. Two commonly used secretagogues include arginine (68, 77, 109, 114, 131) and glucose (70, 82, 122, 128, 140). Under conditions of normal glucose tolerance, the effects of arginine are greater when glucose is infused to maintain an elevated plasma glucose concentration than when arginine is administered at basal glucose (62, 85). The slope of the relationship between the acute insulin response to arginine and the plasma glucose concentration when an arginine bolus is administered at basal glycemia and when glucose is clamped at hyperglycemic levels, is known as the "slope of potentiation" (62). As glucose tolerance deteriorates, the insulin secretory response to arginine diminishes, precipitating a decline in the slope of potentiation of the acute insulin response to arginine.

As discussed previously in this thesis, McCulloch et al. has advocated the slope of potentiation as being the most sensitive in vivo indicator of beta cell function (98). This conclusion is based on an investigation performed in streptozotocin treated baboons. At one week post drug treatment the animals displayed a 94% decrease in the slope of potentiation, while the acute insulin response to glucose and the acute insulin response to arginine were only decreased by 62% and 52%, respectively. In opposition to McCulloch et al.'s conclusion, an investigation performed in normal and islet transplanted canines suggests that the acute insulin response to glucose from the FSIGT is comparable to the slope of potentiation as an indicator of beta cell function (133).

The third specific aim of this thesis was to determine the relationship between the acute insulin response to glucose (AIRg) from the exogenous insulin modified FSIGT and the slope of glucose potentiation of the acute insulin response to arginine (AIRarg), over a wide range of beta cell function. A significant correlation between these two measures of beta cell function, across the spectrum of beta cell function, would allow the AIRg from the FSIGT to be used with confidence as a measure of insulin secretory capacity. This would be advantageous, since the FSIGT also provides data which can be used to determine insulin sensitivity, glucose effectiveness, and glucose tolerance. In addition, the FSIGT protocol is easier and less costly to perform than the slope of potentiation protocol.

Glucose vs. Arginine Stimulation

By performing the FSIGT and the slope of potentiation protocol subsequent to each drug dose in the repeated low dose streptozotocin model, insulin secretory capacity could be assessed longitudinally across a range of beta cell function in canines, from 100% down to 7 - 14% of total capacity. Five measures of insulin secretion were compared: the IIRg and the AIRg from the FSIGT, the AIRarg at basal glycemia, the AIRarg at hyperglycemia, and the slope of potentiation of the acute insulin response to arginine. In contrast to the results of McCulloch et al., results from the present study demonstrate that the acute insulin response to glucose, as well as the integrated insulin response to glucose, significantly correlate with all measures of insulin secretion derived from the slope of potentiation protocol. Furthermore, these correlations are consistent over the entire spectrum of beta cell function. Based on these results I would recommend the AIRg as a reliable measure of beta cell function in dogs.

Since the AIRg is a more common measure of insulin secretion than the IIRg, the focus of the third aim of this thesis project was to validate the use of the AIRg as a reliable measure of beta cell function. Results from the present investigation, however, have demonstrated an even greater correlation between the IIRg from the FSIGT and the slope of potentiation of the AIRarg. Hence, the integrated insulin response to glucose is also recommended as a valid measure of beta cell function.

Though it is not clear why results from the present study and from McCulloch et al.'s study (98) are not compatible, some of the discord could potentially be derived from species differences as well as inconsistencies between experimental protocols.

Species Differences

An investigation performed by Ward et al. (144) provides evidence to dispute the possibility that species differences are responsible for the conflicting results between the present study and McCulloch et al.'s study. Instead of using streptozotocin treated baboons, Ward and colleagues used two-thirds pancreatectomized dogs and also maintain that the slope of potentiation is the best method to detect subclinical beta cell loss. The appropriate question to ask then is, why did Ward et al.'s results agree with those of McCulloch et al.? The answer may lie in the fact that both investigators used similar protocols in their studies.

Protocol Differences

In the protocol used by Ward and colleagues, arginine was injected 4 times for determination of the AIRarg (at 0, 75, 260, and 335 minutes) and glucose was injected at 280 minutes for determination of the AIRg (144). McCulloch and colleagues also combined the glucose tolerance test and the slope of potentiation into one protocol, performed in a single day (98). In their protocol, the glucose injection for determination of the AIRg was administered 30 minutes following the initial arginine injection used to determine the AIRarg at basal glycemia. The second arginine injection for determination of the AIRarg at hyperglycemia was injected approximately 3 hours after the initial arginine injection and approximately 2 1/2 hours after the glucose injection. In comparison, in this thesis investigation, the slope of potentiation protocol was performed two days subsequent to the FSIGT protocol used to determine the AIRg.

Perhaps a residual effect from the prior arginine and glucose injections in McCulloch et al. and Ward et al.'s studies, induced beta cell "resistance" to the potentiating effects of glucose during the AIRarg at hyperglycemia (23). This is based on the concept that the slope of potentiation represents both an overall capacity of the pancreas to secrete insulin and the sensitivity of the beta cells to respond to the potentiating effects of glucose (38). That is, diminished beta cell "sensitivity" to glucose may exist without an impairment in the maximal insulin secretory capacity (75). If such an effect does exist, thereby erroneously reducing the AIRarg at hyperglycemia, an exaggerated decrease in the slope of potentiation would also be exhibited. This could potentially account for the discrepancy between their results and the results from the present study.

Summary

Results from this thesis investigation indicate that the insulin secretory responses to IV glucose and arginine are comparable over a range of beta cell function (approximately 7 - 100%). These results are consistent with those obtained in pancreatectomized/islet transplanted dogs (133). Since the present study confirms this relationship over the spectrum of beta cell function, the IIRg and the AIRg from the FSIGT can now be used as reliable measures of insulin secretion in canines. Validation of the insulin response to glucose as a measure of insulin secretion is important since, the FSIGT protocol not only provides a means to measure this parameter, but also allows the determination of insulin sensitivity, glucose effectiveness, and glucose tolerance. In contrast, the slope of potentiation protocol only provides a measure of beta cell function. Additionally, the FSIGT is more economical and is easier to perform than the slope of potentiation protocol.

Retained Arginine Response

The final issue to be addressed in this discussion regards the literature documenting the presence of a blunted or absent insulin secretory response to IV glucose in the face of a preserved response to other secretogogues, during the initial stages of diabetes. This phenomenon has been documented in both humans (54, 126) and animals (26, 56, 147). An early investigation by Penhos et al. performed in situ via perfusion of the pancreas/small intestine, indicated that higher insulin concentrations are evoked by oral glucose than by IV glucose (111). Investigations since then, have confirmed that when the insulin response to IV glucose is blunted or even absent, the response to oral glucose and non-glucose secretogogues, including arginine, may be retained (26, 54, 56, 126, 147).

This phenomenon is in direct conflict with the results of both McCulloch et al. (98) and Ward et al. (144), where the slope of potentiation (which is dependent upon the acute insulin responses to arginine) was impaired to a greater extent than the acute insulin response to IV glucose. Though results from the present investigation do not concur with those of McCulloch et al. (98) or Ward et al. (144), neither do they support the occurrence of a selective deterioration of the insulin response to IV glucose paralleling a preserved response to arginine. Instead, in the repeated low dose streptozotocin model, the insulin response to IV glucose displayed a significant correlation with the AIRarg at basal glycemia, the AIRarg at hyperglycemia, and the slope of potentiation, throughout the entire study.

One might hypothesize that in the present study, the reason the insulin responses to IV glucose and arginine were not significantly different, is due to some factor inherent in the repeated low dose streptozotocin model. This, however, is probably not the case, since Tobin et al. demonstrated the same phenomenon in islet autografted dogs with only 9 - 29% of insulin secretory function (133).

It is unclear why the diversity exists with respect to the documented trends in the deterioration of the insulin secretory responses to IV glucose and other non-glucose secretogogues. I will address three effects which could potentially be associated with or be responsible for the phenomenon of a lack of response to IV glucose in the presence of a preserved response to arginine: 1) the effect of hyperglycemia, 2) a defect in glucose metabolism, and 3) a defect in insulin synthesis.

Effect of Hyperglycemia

Since the existence of a preserved response to arginine with the simultaneous loss of response to IV glucose has been documented in conditions of both normal fasting blood glucose (54, 126) and mild to moderate hyperglycemia (26, 56, 147), the discord between the various studies does not appear to be related to fasting plasma glucose concentrations. Thus, a "toxic" effect of glucose (i.e. impairment of glucose induced insulin secretion secondary to hyperglycemia) does not seem to be responsible for the blunted insulin response to IV glucose which has been demonstrated during the initial stages of diabetes.

Defect in Glucose Metabolism

Investigations performed by Giroix et al. (56) and Orland and Permutt (105) might provide some insight into this enigma. Giroix et al. examined, in addition to other stimuli, the effects of glucose and arginine on insulin secretion in perfused pancreas from streptozotocin treated rats (56). Glyceraldehyde, a metabolite of glucose metabolism, was also studied to determine it's effects upon insulin secretion in the absence of glucose. In the diabetic pancreas, glyceraldehyde stimulated insulin secretion to a similar extent as in the control pancreas. During exposure to glucose, however, the insulin response was absent, but the response to arginine was preserved. Based on these results, they suggest that the reason for the lack of response to glucose may involve a block in beta cell glucose metabolism (the glycolytic pathway) prior to the triose phosphate (glyceraldehyde) level. This supports the premise that the metabolism of glucose within the beta cell is involved in the normal process of glucose recognition or the hormonal response it induces (34, 64, 93, 156). With regards to the present study, if Giroix et al.'s hypothesis is true, the repeated low dose streptozotocin model in canines does not induce the same defect in glucose metabolism that has been observed in previous investigations in which the insulin response to glucose is absent, while the response to arginine is maintained.

Defect in Insulin Synthesis

In light of Giroix et al.'s hypothesis (56) and a recent study by Orland and Permutt (105), a defect in the metabolism of glucose within the beta cell (56) could potentially prevent normal insulin synthesis (105), thereby resulting in a depressed or abolished insulin secretory response. In Orland and Permutt's investigation (105), insulin synthesis and secretion were determined in 4 groups of rats: 1) pancreatectomized (50%), 2) sham controls, 3) pancreatectomized / dexamethasone (a drug which induces insulin resistance) treated, and 4) sham / dexamethasone treated. Insulin synthesis was estimated by measuring pancreatic proinsulin mRNA and the acute insulin response to arginine (AIRarg) was used as an indicator of insulin secretion. A significant correlation was found between the AIRarg (insulin secretion) and the pancreatic proinsulin mRNA content (insulin synthesis). These results suggest that a reduced insulin secretory response may be associated with a defect in insulin synthesis. With regards to the issue of an attenuated insulin response to IV glucose in the presence of a preserved response to arginine, it may be that a defect lies at some point in the cascade of events (e.g. a block in beta cell glucose metabolism (56)), which prevents normal glucose stimulated insulin synthesis and ultimately, secretion. Since both the insulin responses to glucose and arginine are maintained in the present study, it may be possible that such a defect in glucose metabolism is not present in the repeated low dose streptozotocin model. This metabolic difference may therefore, contribute to the heterogeneity between this model of diabetes and true IDDM, as well as between this model and other animal models of diabetes.

Summary

Though some data suggests that the insulin secretory response to arginine may be preserved even when the insulin response to IV glucose is abolished, this phenomenon was not displayed in the present study. The reason for the inconsistency is not known, though it may be related to the heterogeneity between this animal model of subclinical insulin deficiency and actual IDDM or other models of diabetes. Evidence does support the hypothesis that the apparent abnormality in IV glucose stimulated insulin secretion may not reflect beta cell secretory capacity, but instead may represent a defect in beta cell glucose metabolism. Further research would be required to determine the basis for the incongruous insulin secretory responses to IV glucose and non-glucose secretogogues and to determine the cause for the dissimilarities between studies with regards to this phenomenon.

D. GENERAL SUMMARY

By administering 10 low doses (10 mg/kg) of streptozotocin at 14 day intervals to dogs, an animal model of subclinical insulin deficiency was established. Using this novel model of diminished insulin secretory function, it has been demonstrated that despite an 86 - 93% reduction in the insulin secretory response, fasting plasma insulin, fasting plasma glucose, and insulin sensitivity did not change. Glucose effectiveness, however, declined to 45% of pre-treatment values by completion of the study.

These results support the theory that a reduction in insulin sensitivity is not a direct consequence of beta cell dysfunction. In contrast, the observed defect in glucose effectiveness may be secondary to chronic insulin deficiency. Furthermore, the impairment in glucose effectiveness may contribute to the glucose intolerance observed during subclinical insulin deficiency.

In addition, it has been established that the integrated and the acute insulin responses to glucose from the FSIGT are equivalent to the slope of potentiation of the acute insulin response to arginine as measures for quantification of beta cell function in dogs. Significant correlations were demonstrated between these in vivo measures of beta cell function over a range of approximately 7 - 100% insulin secretory capacity. Thus, both the integrated and the acute insulin responses to glucose from the FSIGT can now be used as reliable measures of beta cell function in dogs.

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